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ORIGINAL INVESTIGATION

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Tacrine-scopolamine interactions on state-dependent retrieval

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Abstract *Objectives:* This study examined the effects of tacrine on scopolamine-induced state-dependence. *Methods:* Rats were trained to complete an FR10 schedule of lever presses for milk reward within 120 s after the onset of an operant session and were subsequently tested for the retrieval of the response in either the same or a different, pharmacologically defined, state. *Results:* In rats trained with 2.5 mg/kg scopolamine, the pre-test administration of 10 mg/kg tacrine prevented scopolamine from enabling the retrieval that otherwise occurred when animals were both trained and tested with scopolamine. However, retrieval of the response was also hampered in animals that were trained with tacrine-scopolamine co-administration and tested with saline, and vice versa, indicating that the co-administration of tacrine and scopolamine did not induce the saline-associated, presumably normal state. At ≥2.5 mg/kg doses, tacrine itself induced state-dependence with both tacrine-to-saline and saline-to-tacrine state changes. *Conclusion:* The findings indicate that tacrine is unable to normalize the particular mnesic state induced by scopolamine. The data may elucidate tacrine's limited therapeutic efficacy insofar as scopolamine's mnesic actions both model human pathology and are due to scopolamine producing state-dependence.

Keywords Tacrine · Scopolamine · State dependence · Retrieval · Rats · Memory

Introduction

Memory function presumably comprises different processes that include learning, encoding, retention and retrieval (Tulving 1983; Pearce 1987; Sekiguchi et al. 1997; Milner et al. 1998). Deficits in one or several of these processes appear to be responsible for the mnesic decline that emerges in Alzheimer's disease (AD; Whitehouse et al. 1981; Kopelman and Corn 1988). In addition to brain lesions and genetic manipulations, models of mnesic dysfunction are provided (McDonald and Overmier 1998) by centrally acting compounds; well-characterized among these agents are ketamine (Krystal et al. 1994), chlordiazepoxide (Golombok et al. 1988; Curran 1991), ethanol (Maylor and Rabbitt 1987), and, especially, the muscarinic acetylcholine receptor antagonist scopolamine (Caine et al. 1981; Broks et al. 1988; Kopelman and Corn 1988; Rusted and Warburton 1988). Scopolamine is of particular interest with regard to the study of AD, since the latter is characterized by a large and progressively worsening deficit in cholinergic activity (e.g. Bartus 2000).

Memory can be state-dependent in that a response that has been acquired in a given (e.g. drug-induced) state may not be retrieved when the organism is in a different state. We have recently confirmed earlier evidence (e.g. Berger and Stein 1969; Pert and Avis 1974) showing scopolamine to sustain state-dependence (StD) in rats (Colpaert et al. 2001). Indeed, both saline-to-scopolamine and scopolamine-to-saline changes of state caused large decrements in the retrieval of a milk-rewarded operant lever press response. Remarkably, while the saline- or scopolamine-induced states exerted few or no intrinsic effects on the different mnesic processes, changes of state severely hampered not only retrieval, but also learning, encoding and retention (Colpaert et al. 2001). For example, the learning of a response was disrupted when the latter had initially been acquired in the scopolamine state and was further learned in the saline state. Also, changing the state as the trace was being encoded, post-session, acted to impair the session-to-session carry-over of learning effect and thus abolished acquisition. These and other findings led us to suggest that state changes induced by excessively labile cholinergic neurotransmission may operate endogenously and perhaps constitute the mechanism of the mnesic deficits seen in AD (Colpaert et al. 2001).

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Different theories and therapeutic approaches of AD are currently being pursued (e.g. antioxidant, neurotrophic or anti-amyloid strategies; see Small 1998). However, based on the hypothesis that the mnesic and cognitive decline in AD is due to the deterioration of cholinergic transmission, the major focus of the treatment of AD is on the increase or prolongation of the effects of remaining acetylcholine (Bartus et al. 1982; but see Baxter and Chiba 1999; Davis et al. 1999). The reversible acetylcholinesterase inhibitor (AChEI), tacrine (9-amino-1,2,3,4-tetrahydroacridine; Shaw and Bentley 1953) acts to enhance the action of endogenous acetylcholine at cholinergic receptor sites by blocking acetylcholine hydrolysis by acetylcholinesterase. Numerous behavioral studies have shown beneficial effects of AChEI in animals with cholinergic deficits. For example, in rats, tacrine antagonized scopolamine-induced memory impairments in a radial arm maze (M'Harzi et al. 1995; Ogura et al. 2000) and Morris water maze task (Wang et al. 1999), improved passive-avoidance retention deficits induced by both aging and medial septal lesions (Riekkinen et al. 1991) and reversed AF64A-induced working memory deficits (Cheng and Tang 1998); in rhesus monkeys, tacrine reversed the scopolamine-induced response decrease on a continuous performance task (Callahan 1999).

The present studies examined the effects of an AChEI on scopolamine-induced StD. While improved AchEIs have since become available (e.g. Dronfield et al. 2000), tacrine was used because it was best characterized at the time, now a decade ago, when these studies were initiated. Specifically, and since scopolamine-induced StD may help to understand the mechanisms underlying disordered memory (Colpaert et al. 2001), we here determined, using a milk-rewarded operant lever press response, whether tacrine could counteract the decrement in retrieval that occurs when rats acquire this response with scopolamine and are tested for retrieval with saline, and vice versa. We further investigated the tacrine-scopolamine interaction by determining whether the co-administration of scopolamine and tacrine can induce a saline-like, presumably normal state of memory. Finally, we determined whether tacrine could itself sustain state-dependence. The experimental conditions and scopolamine dose (i.e. 2.5 mg/kg) that were implemented here, were identical to those with which previous work indicated scopolamine to act solely by inducing a mnemonic state, while exerting no marked intrinsic effects (on learning, encoding, retention, retrieval; Colpaert et al. 2001). The doses of tacrine used (i.e. 0.04–10 mg/kg) were similar to those at which tacrine produced dose-dependent effects in other studies of memory (e.g. Chopin et al. 2002). Finally, it is useful to point out that unlike other procedures often employed in the study of memory (e.g. the water maze; Morris et al. 1982), the state-dependence paradigm used here implements lever pressing as the dependent variable. Like many other procedures however, the paradigm uses response latency as a measure of retrieval. Note that this latency measure appears to be confounded little, it at all, by the discriminative effects, nor by the effects on response rate, that various pharmacological agents may also produce (Colpaert 1990; Colpaert and Koek 1995; Bruins Slot and Colpaert 1999b).

Materials and methods

Subjects

Male Sprague-Dawley rats (Iffa Credo, Lyon, France) weighing 180–200 g on arrival were used. Upon arrival and for a quarantine period of 4–5 days, animals were housed five to a cage in an environmentally controlled room (ambient temperature, 21±1°C; relative humidity, 55±5%; 12:12 h light:dark cycle, lights on 0700 hours) with standard laboratory food and water freely available. The rats were transferred to the experimental room on the day before experiments began and maintained under the same environmental conditions as during quarantine. Access to food was then limited to 20 g per day, except between Friday 1700 hours and Sunday 1400 hours, when food was available freely. The protocol was in accordance with "Principles of laboratory animal care" (NIH publication 85-23, revised 1985) and approved by the institutional Ethical Review Committee (No. 009).

Operant apparatus and procedures

The experimental apparatus as well as the acquisition and test procedures were similar to those described elsewhere (Bruins Slot et al. 1999). Briefly, the apparatus consisted of operant conditioning chambers, housed in fan-ventilated, light-and-sound attenuating enclosures. Each chamber contained a house-light, a lever and a liquid dipper. Reinforcement consisted of a 4-s access to the liquid dipper that contained 0.02 ml of sweetened condensed milk.

Rats were randomly assigned to the operant chambers in which they were trained to lever press for access to milk during daily 15-min sessions until they could complete an FR10 schedule of lever-presses for the milk reward. Before each of these acquisition sessions, the animals received a pharmacological treatment that remained the same for individual animals throughout acquisition. Training continued until animals completed the first FR10 within 120 s after the beginning of the session. Animals that had not reached this criterion performance after 40 sessions were discarded. A period of 48 h was allowed to elapse between the criterion session (the acquisition session during which animals reached the criterion performance) and the test session. On the day of the test session animals received a pharmacological treatment that was either the same (i.e. "same-state") or different (i.e. "changed-state") from the one implemented during acquisition and were tested for the retrieval of the response during a single 15-min test session. Animals were tested only once.

Single treatments were administered subcutaneously (SC) 60 min before the session; double treatments were administered SC 60 min before and intraperitoneally (IP) 30 min before the beginning of the session (injection volume: 1 ml/100 g body weight).

Experimental design

In the first series of experiments, animals were trained to criterion with saline and scopolamine (2.5 mg/kg) and were then tested for retrieval with one of different doses of tacrine (i.e. 0.63, 1.25, 2.5, 5 or 10 mg/kg) and scopolamine (2.5 mg/kg) (n=7–8 per dose of tacrine). Same-state controls animals were both trained and tested with two injections of saline (same-state saline controls; n=25) or trained and tested with saline and 2.5 mg/kg scopolamine (same-state drug controls; n=14). As these experiments demonstrated an inhibition of the scopolamine state by tacrine, we further investi-

gated whether a co-treatment with tacrine and scopolamine could induce a saline-like state. Thus, in the second series of experiments, animals were trained with one of different doses of tacrine (0.04, 0.16 or 0.63 mg/kg) and scopolamine (2.5 mg/kg) and tested with two saline injections (n=8–10 per dose of tacrine). Controls were trained with saline and 2.5 mg/kg scopolamine and tested with two injections of saline (changed-state controls; n=23) or trained and tested with tacrine (0.16 mg/kg) and 2.5 mg/kg scopolamine (same-state controls; n=12). Other groups were trained with saline and saline and tested with tacrine (0.16, 0.63, 1.25, 2.5, 5 or 10 mg/kg) and scopolamine (2.5 mg/kg) (n=9–10 per dose of tacrine). Further controls consisted of animals trained with two injections of saline and tested with saline and 2.5 mg/kg scopolamine (changed-state controls; n=26).

The experiments that are specified above did not find tacrine to normalize the scopolamine-induced mnesic state. Thus, two further series of experiments were conducted to investigate whether tacrine can in itself induce StD. In the first series, rats were trained to criterion with tacrine (0.63, 1.25 or 2.5 mg/kg) and tested with saline (n=7–10 per dose of tacrine). In the second series of experiments, animals acquired the response with saline and were tested with tacrine (0.63, 1.25 or 2.5 mg/kg) (n=7 per dose of tacrine). For both series, control groups consisted of animals trained and tested with saline (same-state saline controls; n=25). Further controls consisted of animals that were both trained and tested with 2.5 mg/kg tacrine (same-state tacrine controls; n=13).

Drugs

Tacrine and (–)scopolamine hydrobromide trihydrate (Merck; Nogent-sur-Marne, France) were used. The two compounds were dissolved in distilled water; doses refer to the free base weight.

Data analysis

The data analyzed (see Bruins Slot and Colpaert 1999a) were the number of sessions to reach criterion, and the latency to complete the first FR10 during the criterion and the test sessions. Sessions-to-criterion (STC) values were analyzed non-parametrically by means of the Kruskall-Wallis analysis of variance on ranks (Siegel and Castellan 1988); where this analysis yielded significance, post-hoc comparisons were carried out using a non-parametric equivalent of the Dunnett's test for multiple comparisons (Zar 1984). After a normalizing log-transformation, latency ratios (i.e. the ratio of latency to complete the first FR10 during the criterion session to the latency to complete the first FR10 during the test session) were analyzed parametrically by means of ANOVA (Winer 1971); where this analysis yielded significance, post-hoc comparisons were carried out using Dunnett's test for multiple comparisons. Other comparisons used the Student's t-test to determine differences in means between two experimental groups. ED_{50} values and 95% confidence limits were computed according to the method of Litchfield and Wilcoxon using the PHARM/PCS program of Tallarida and Murray (1987). Statistical significance was defined as P<0.05.

Results

Drug effects on acquisition

In the different experiments reported below, only one out of in all 143 animals (i.e. 0.7 or <5%) that were trained with two saline injections, failed to reach criterion in less than 40 sessions. Criterion was not reached in less than 40 sessions in 29 out of 103 of the animals (i.e. 28%) trained with saline and scopolamine (2.5 mg/kg). Animals trained with different doses of tacrine (i.e. 0.63 to 5 mg/kg) and saline also showed impaired acquisition; based on the percentage of animals reaching criterion within 40 sessions, the ED_{50} (and 95% CL) for tacrine to impair acquisition was 2.7 (0.31–24) mg/kg. Furthermore, acquisition was impaired in animals trained with different doses of tacrine (i.e. 0.04–2.5 mg/kg) and scopolamine (2.5 mg/kg); the ED_{50} for tacrine to impair acquisition in the presence of scopolamine was 0.24 (0.011–5.3) mg/kg. A comparison using the PHARM/PCS program failed to find a significant difference in these ED_{50}s (P>0.05); thus, tacrine certainly did not improve scopolamine's effects on acquisition.

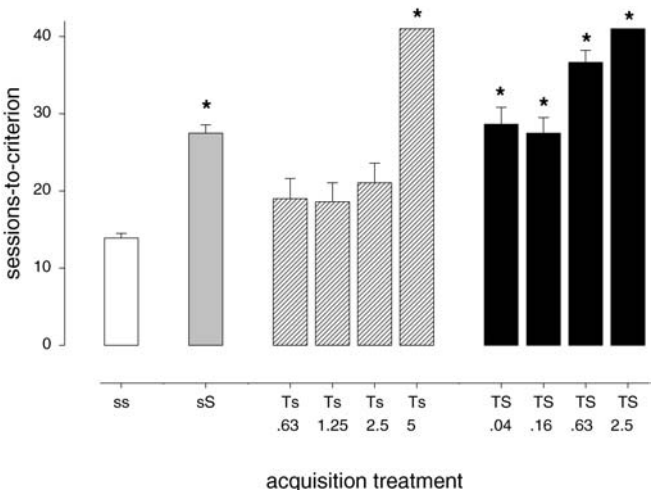

Fig. 1 Drug effects on acquisition. Rats were trained in a milk-rewarded lever pressing task to complete a fixed-ratio 10 (FR10) schedule within the first 120 s of a 15-min session. During acquisition, one of the following pharmacological treatments was administered: two injections of saline (*ss*); saline and scopolamine 2.5 mg/kg (*sS*); different doses of tacrine (0.63–5 mg/kg) and saline (*Ts*); or different doses of tacrine (0.04–2.5 mg/kg) and scopolamine 2.5 mg/kg (*TS*). The two injections were given SC 60 min before and IP 30 min before the beginning of the training sessions. Ordinates represent the number of sessions-to-criterion (mean±SEM; cut-off: 40 sessions) that expired in acquisition before a session occurred in which the FR10 schedule was completed within 120 s

An overall analysis of the STC data (Fig. 1) by means of a Kruskall-Wallis analysis of variance on ranks revealed a significant effect (P<0.001) of acquisition treatment; post-hoc comparison with saline controls showed significant differences (P<0.05) to occur for animals trained with saline and scopolamine (2.5 mg/kg), animals trained with 5 mg/kg tacrine and saline, and animals trained with different doses of tacrine (0.04–2.5 mg/kg) and scopolamine (2.5 mg/kg).

Can tacrine antagonize scopolamine at the time of retrieval in scopolamine-trained animals?

Same-state drug controls that were both trained and tested with a treatment consisting of saline and 2.5 mg/kg

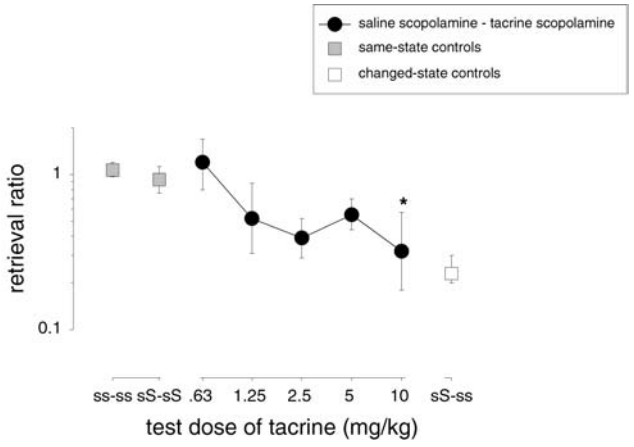

Fig. 2 Effects of tacrine during test in scopolamine-trained animals. Training sessions occurred after two injections of saline and 2.5 mg/kg scopolamine. Once trained, animals were tested during a single test session with different doses of tacrine (*abscissa*) and 2.5 mg/kg scopolamine (*black circles*). Same-state saline controls (*ss-ss*) were trained and tested with saline while same-state drug controls (*sS-sS*) were trained and tested with saline and 2.5 mg/kg scopolamine (*grey squares* in either case); changed-state controls (*white squares*: *sS-ss*) were trained with saline and 2.5 mg/kg scopolamine and tested with two saline injections. The double treatments were administered SC 60 min before and IP 30 min before the beginning of the training sessions. Ordinates represent the log-transformed ratios (i.e. the ratio of the latency found in the criterion session to the latency found in the test session; geometric mean±SEM). Multiple comparisons using Dunnett's method: **P*<0.05

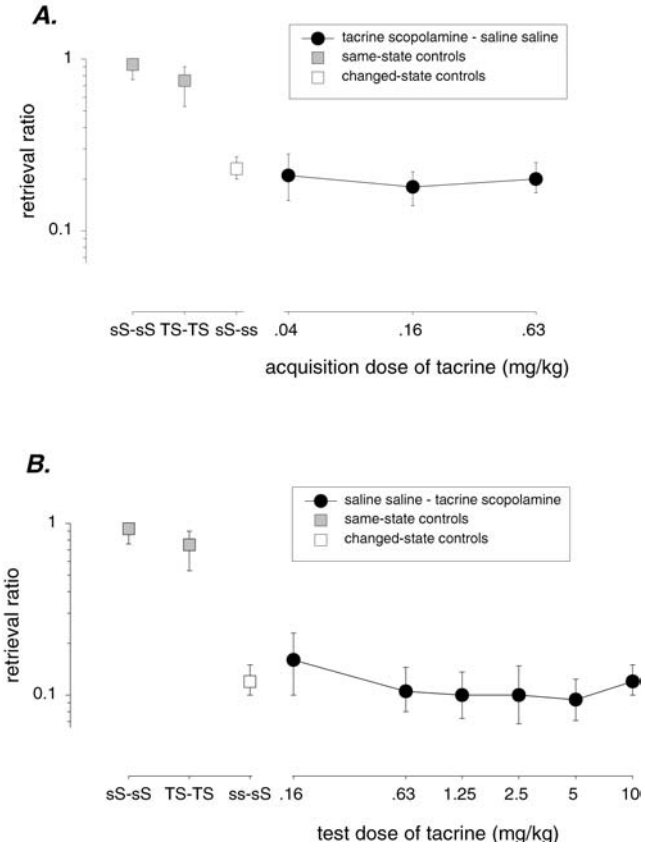

Fig. 3A,B Comparison between the state induced by the co-administration of tacrine and scopolamine and that induced by saline. **A** Training sessions occurred after two injections of different doses of tacrine (*abscissa*) and 2.5 mg/kg scopolamine. Once trained, animals were tested during a single test session with two injections of saline (*black circles*). Same-state controls were trained and tested with saline and 2.5 mg/kg scopolamine (*sS-sS*) or trained and tested with 0.16 mg/kg tacrine and 2.5 mg/kg scopolamine (*TS-TS*) (*grey squares*); changed-state controls (*sS-ss*) were trained with saline and 2.5 mg/kg scopolamine and tested with two saline injections (*white squares*). **B** Training occurred after two saline injections. Once trained, animals were tested during a single test session with different doses of tacrine (*abscissa*) and 2.5 mg/kg scopolamine (*black circles*). Same-state controls as above; changed-state controls (*ss-sS*) were trained with two saline injections and tested with saline and 2.5 mg/kg scopolamine (*white squares*). The double treatments were administered SC 60 min before and IP 30 min before the beginning of the training sessions. Ordinates represent the log-transformed ratios (i.e. the ratio of the latency found in the criterion session to the latency found in the test session; geometric mean±SEM)

scopolamine demonstrated retrieval ratios of about 1 (Fig. 2), indicating an adequate retrieval of the response acquired during training. Similar retrieval ratios were obtained in same-state saline controls that were trained and tested with two saline injections; retrieval ratios did not significantly differ between these two same-state control groups (Student's *t*-test, $P>0.05$). In contrast, changed-state controls that were trained with saline and 2.5 mg/kg scopolamine and tested with two saline injections showed a large impairment in the retrieval of the response; retrieval ratios in these changed-state controls were considerably lower than those in same-state drug controls (Student's *t*-test, $P<0.001$).

The co-administration at the time of test of different doses of tacrine (i.e. 0.63–10 mg/kg) with 2.5 mg/kg scopolamine in animals trained with saline and 2.5 mg/kg scopolamine, thus significantly impaired retrieval [ANOVA, pre-treatment effect, $F(5,43)=2.5$; $P<0.05$]. Compared to the same-state controls that were both trained and tested with saline and 2.5 mg/kg scopolamine, post-hoc comparisons showed a significant effect to occur with 10 mg/kg tacrine (Dunnett's test, $P<0.05$; Fig. 2).

Does the co-administration of tacrine and scopolamine induce a saline-like state?

Acquisition with tacrine and scopolamine

Animals that were trained with saline and 2.5 mg/kg scopolamine and tested with two injections of saline (changed-state controls) showed a large impairment in the retrieval of the response, the (geometric) mean retrieval ratio being 0.23 (0.20–0.27) (Fig. 3A). In contrast, same-state controls that were trained and tested with 0.16 mg/kg

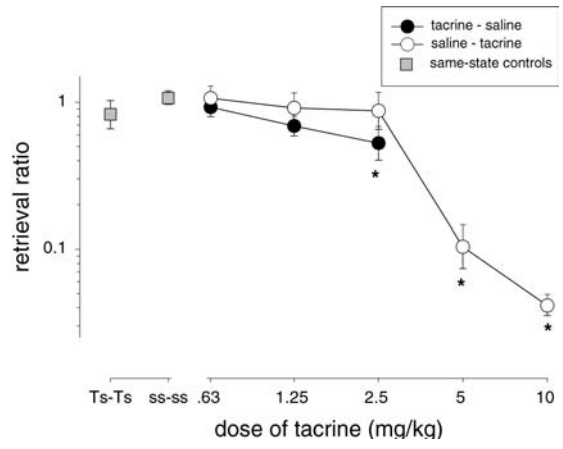

Fig. 4 Tacrine-induced state-dependence. Animals were either trained with different doses of tacrine and tested with saline (*black circles*; *tacrine-saline*) or trained with saline and tested with different doses of tacrine (*white circles*; *saline-tacrine*). Same-state drug controls were trained and tested with 2.5 mg/kg tacrine (*Ts-Ts*) and same-state saline controls were trained and tested with saline (*ss-ss*) (*grey squares*). Treatments were administered SC 60 min before the sessions. *Ordinate* represent the log-transformed ratios (i.e. the ratio of the latency found in the criterion session to the latency found in the test session; geometric mean±SEM). Multiple comparisons using Dunnett's method: **P*<0.05

tacrine and 2.5 mg/kg scopolamine demonstrated retrieval ratios close to 1, indicating again an adequate retrieval of the response in same-state conditions. Animals that were trained with one of several doses of tacrine (i.e. 0.04–0.63 mg/kg) and 2.5 mg/kg scopolamine and tested with two injections of saline also showed an impairment in the retrieval of the response that was large and similar to that observed in changed-state controls [ANOVA, pre-treatment effect, $F(3,46)=0.29$; $P=0.83$]. Higher acquisition doses of tacrine in the presence of 2.5 mg/kg scopolamine could not be examined due to deleterious effects on acquisition (Fig. 1).

Testing with tacrine and scopolamine

Control animals that were trained with two injections of saline and tested with saline and 2.5 mg/kg scopolamine (changed-state controls) showed a large impairment in the retrieval of the response, the mean retrieval ratio being 0.12 (0.10–0.15) (Fig. 3B). Animals that were trained with two injections of saline and tested with one of several doses of tacrine (i.e. 0.16–10 mg/kg) and 2.5 mg/kg scopolamine also showed an impairment in the retrieval of the response that was large and similar to that observed in changed-state controls [ANOVA, pre-treatment effect, $F(6,74)=0.31$; $P=0.93$].

Tacrine-induced state-dependence

Animals that were trained and tested with saline (same-state saline controls) or trained and tested with 2.5 mg/kg tacrine (same-state drug controls) demonstrated similar (Student's *t*-test, $P>0.05$) retrieval ratios of about 1 (Fig. 4), again indicating retrieval to be adequate in same-state conditions. However, significant impairment of retrieval occurred when animals trained with tacrine (i.e. 0.63, 1.25 or 2.5 mg/kg) were tested in the normal state (i.e. after saline injection) [ANOVA, treatment effect, $F(3,45)=4.1$, $P<0.05$]; post-hoc comparisons revealed a significant difference for the 2.5 mg/kg acquisition dose. Higher acquisition doses could not be examined due to their effects on learning (Fig. 1).

Animals that were trained with saline and tested with one of several test doses of tacrine (i.e. 0.63, 1.25, 2.5, 5 or 10 mg/kg) also showed a dose-dependent impairment of retrieval [ANOVA, treatment effect, $F(5,54)=44.7$, $P<0.001$], this effect being significant at the 5 and 10 mg/kg test doses (Dunnett's test, $P<0.05$).

Discussion

The present studies examined the effects of tacrine on scopolamine-induced state-dependence; a response was established in a given pharmacological state and tests of retrieval were conducted in either the same or a different state. For this purpose, rats were trained to complete an FR10 schedule of lever presses for milk reward within 120 s after the onset of an operant session. The results obtained in the tacrine-scopolamine interaction experiments are in accordance with previous findings (M'Harzi et al. 1995; Callahan 1999; Wang et al. 1999; Ogura et al. 2000), inasmuch as they indicate tacrine to counteract the effects of scopolamine in laboratory animals. That is, in scopolamine-trained rats, the pre-test administration of tacrine prevented scopolamine from enabling the retrieval that otherwise occurred when so-trained animals were tested with scopolamine alone (Fig. 2). In a further series of studies, we examined whether the co-administration of tacrine and scopolamine could induce a state similar to the presumably normal state that is associated with the injection of saline. This appeared not to be the case; retrieval of the response was completely abolished in animals that were trained with co-administration of tacrine and scopolamine and tested with two saline injections (Fig. 3A). Similarly, animals trained with two saline injections and tested with co-administration of tacrine and scopolamine also showed a significant impairment in the retrieval of the response (Fig. 3B).

The outcome of these experiments examining tacrine-scopolamine interactions was unlike that obtained in similarly designed studies examining interactions between agents acting at a same molecular site (Bruins Slot and Colpaert 1999b). That is, pretreatment with the μ-opioid antagonist naloxone prevented the μ-opioid agonist morphine from enabling the retrieval that otherwise occurred when animals were both trained and tested with morphine alone. However, adequate retrieval did occur in saline-trained animals that were tested with either naloxone or after the co-administration of naloxone and

morphine. Thus, and unlike tacrine-scopolamine co-administration, naloxone-morphine co-administration appeared to be capable of resulting in a saline-like state. One possible reason for the failure of tacrine-scopolamine co-administration to produce a saline-like state is that tacrine may itself produce a mnesic state. This possibility was supported by the finding that tacrine dose-dependently impaired retrieval in animals trained with saline (Fig. 4). Also, animals trained with tacrine showed impaired retrieval when tested with saline; the latter effect appeared to be dose-dependent and was significant, but did not reach a large amplitude. However, only doses of tacrine up to 2.5 mg/kg could be examined (Fig. 4) since higher doses severely hampered acquisition (Fig. 1). The molecular sites involved in this tacrine state may be diverse. By elevating endogenous acetylcholine through cholinesterase inhibition, tacrine may indirectly activate not only muscarinic but also facilitatory nicotine cholinoceptors (Loiacono and Mitchelson 1990). This, and further evidence demonstrating AChE-independent actions of tacrine on muscarinic (Hunter et al. 1989; Adem et al. 1990) and nicotinic receptors (Nillson et al. 1987; Clarke et al. 1994) suggests that tacrine may exert molecular and cellular actions involving other than scopolamine-sensitive sites.

The present findings may be of interest in view of the apparent disparity between animal studies typically demonstrating tacrine to robustly counteract memory-impairing effects of scopolamine, and clinical studies finding tacrine effects on cognitive function to be only modest and confined to a minority of patients (e.g. Davis et al. 1992; see Malaguernera 1998 for meta-analysis). The data in Fig. 2 are consistent with earlier evidence (M'Harzi et al. 1995; Callahan 1999; Wang et al. 1999; Ogura et al. 2000) inasmuch as they demonstrate that tacrine can counteract at least some cognitive actions of scopolamine. However, further StD analysis indicates that in the presence of scopolamine, tacrine fails to normalize the state of memory that otherwise enables retrieval (Fig. 3). The latter failure appears to be due to tacrine itself producing a mnesic state that differs from both the normal state (Fig. 4) and that induced by scopolamine (Fig. 2). Previous StD research (Colpaert et al. 2001) would suggest that because of these features, tacrine may in fact impair cognitive performance as changes of mnesic state may not only impair retrieval, but also perturb learning, encoding and retention. Thus, and consistent with clinical studies, the StD analysis of tacrine's actions may elucidate the compound's limited overall effects on the deficits in different memory functions that make up cognitive disorders. Study designs such as those implemented in earlier research on scopolamine (Colpaert et al. 2001) may help to assess the validity of StD analyses to tacrine's actions in humans.

As indicated above (see Introduction), the present experiments were conducted in conditions in which scopolamine's actions are solely mediated by scopolamine-induced StD; tacrine's failure to normalize performance in these conditions raises the question as to how human mnesic pathology can be remedied if it were due to scopolamine-like StD. As discussed elsewhere (Colpaert et al. 2001), the possible state change induced by disease (e.g. that possibly associated with lowered activation of acetylcholine receptors), may impair retrieval, but this deficit can be overcome by de novo learning and, perhaps very briefly, by tacrine. However, excessive stage changes (state lability), must be expected to not only impair retrieval, but also to devastate learning, encoding and retention, resulting in several intricate disabilities (Colpaert et al. 2001). Future treatments therefore may seek to stabilize, rather than merely enhance (as tacrine may), the activation state of acetylcholine receptors.

Several caveats are in order with the present and earlier (Colpaert et al. 2001) analyses. The neurophysiological nature of the memory trace that is being studied in the paradigm used here remains poorly identified (Colpaert et al. 2001) and in contrast with its actions in this paradigm, scopolamine in other conditions may impair acquisition while apparently failing to produce StD (Elrod and Buccafusco 1988). However, the putative non-occurrence of StD is difficult to ascertain, as retrieval tests may set the occasion for new learning to occur; because of this, and depending on the particular conditions being used, StD effects may appear to last for as little as 1 min, if not a few seconds (Colpaert et al. 2000). Further, tacrine may improve performance at least partially in conditions (e.g. of lesion-induced or age-related impairments) that do not involve scopolamine and in which a role for StD has not been established (e.g. Kirkby and Higgins 1998; Chopin et al. 2002). It is useful to note here, though, that StD effects have been shown to span up to half the rat's life expectancy (Colpaert et al. 2001). Finally, and as with other theories of memory (McDonald and Overmier 1998), the concept of state-dependent memory remains poorly defined and may not at this stage preclude falsifiability.

In conclusion, the present studies indicate that tacrine counteracts scopolamine in producing a state that otherwise enables retrieval in rats that acquired an operant response in a scopolamine-induced state of memory. However, the co-administration of tacrine and scopolamine did not produce a presumably normal, saline-like state; this may be due to the ability of tacrine in itself to induce StD as it caused retrieval deficits with both tacrine-to-saline and saline-to-tacrine state changes. The finding that tacrine itself induces a mnesic state may perhaps elucidate its limited efficacy in clinical studies.

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