Sabcomeline (SB-202026), a functionally selective $M_1$ receptor partial agonist, reverses delay-induced deficits in the T-maze

Abstract  Sabcomeline, (SB-202026 $[R$-($Z$)-$\alpha$-(methoxyimino)-1-azabicyclo [2.2.2] octane-3-acetonitrile]), a functionally selective muscarinic $M_1$ receptor partial agonist, was tested in rats trained to perform a delayed, reinforced alternation task in a T maze, a test of short-term spatial memory. For comparison the cholinesterase inhibitor tacrine (THA-9-amino-1,2,3,4-tetrahydroaminoacridine) and the non-selective muscarinic receptor agonist RS86 (2-ethyl-8-methyl-2,8-diazaspiro [4.5]-decane-1,3-dione hydrobromide) were also tested and all three compounds were also compared using a conditioned taste aversion (CTA) task. Sabcomeline (0.001–1.0 mg/kg IP) significantly reversed the T-maze choice accuracy deficit induced by a 20-s delay at 0.03 and 0.1 mg/kg. RS86 (0.1–3.0 mg/kg IP) reversed the deficit at 1.0 mg/kg and THA (0.1–3.0 mg/kg IP) had no effect at any dose. All three compounds induced conditioned taste aversion with minimum effective doses (MED) of 0.3, 1.0 and 3.0 mg/kg, respectively. The results show that sabcomeline reverses delay induced deficits in T-maze choice accuracy in a rewarded alternation task at doses approximately 10 times lower than those required to induce conditioned taste aversion. RS86 was equipotent in both tests. These data support the findings of clinical studies which have shown that SB-202026 provides significant symptomatic improvement in patients with probable Alzheimer’s disease at doses which do not induce cholinergic side effects.

Key words  Alzheimer’s disease · T-maze · Conditioned taste aversion · Muscarinic agonists · Sabcomeline · SB-202026 · THA · RS86 · Rat

Introduction  Alzheimer’s disease (AD) is characterised by complex pathology, including marked degeneration of the basal forebrain cholinergic neurons which project to the cortex and hippocampus (Davies and Maloney 1976; Coyle et al. 1983). Reductions in presynaptic markers, including choline acetyltransferase (ChAT) (Mash et al. 1985; Hoss et al. 1990), correlate with the severity of the disease (Perry et al. 1978). However, the density of $M_1$ muscarinic receptors, which are mainly postsynaptic, remains largely unchanged (Rinne et al. 1985; Quirion et al. 1989; Svensson et al. 1992). Animal studies suggest that ACh plays a role in learning and memory (Hagan and Morris 1988; Dunnett et al. 1991; Dunnett and Fibiger 1993) and, in addition, $M_1$ muscarinic receptors have been shown to be involved in spatial memory processing (Hagan et al. 1987; Andrews et al. 1994). Therefore, selective $M_1$ muscarinic receptor agonists may provide symptomatic treatment for Alzheimer’s disease.

Muscarinic receptor agonists such as arecoline, RS86, oxotremorine and pilocarpine have limited efficacy in tests of cognition (see Sarter et al. 1992a,b for reviews) but are non-selective for muscarinic receptor subtypes. More selective compounds show greater promise in clinical and pre-clinical studies, for example AF150(S) (Brandeis et al. 1995), L-687,306, AF102B (Dawson and Iversen 1993), BIMC 182 (Cereda et al. 1994), CI-979 (M’Harzi et al. 1995), YM796 (Suzuki et al. 1995). Of the few muscarinic agonists for which clinical tolerance data have been reported, all have caused cholinergic side effects to a varying degree (see Cutler and Sramek 1995 for review). Xanomeline (LY246708 tartrate), a functionally selective muscarinic $M_1$ receptor agonist, caused diarrhoea, nausea and emesis in AD patients at 115 mg t.i.d (Fisher and Barak 1994), and blood pressure and heart rate changes at lower doses (Medina et al. 1997). Milameline (CI-979) caused dose-limiting cholinergic symptoms and parkinsonian behaviours in a study of healthy volunteers and patients with Alzheimer’s disease (Sramek et al. 1995). AF102B, at doses of 40 and 60 mg
t.i.d., caused cognitive improvement and was tolerated by AD patients with diaphoresis and hypersalivation at the higher dose (Fisher et al. 1996). For successful treatment of AD, it is clearly important that the dose of a compound which enhances cognition is lower than that which evokes cholinergic side effects.

Sabcomeline (SB-202026) is the hydrochloride salt of \(R-(Z)-(\pm)-(\text{methoxyimino})\text{-1-azabicyclo [2.2.2] octane-3-acetonitrile}\) (Bromidge et al. 1994). It is a potent \(M_1\) partial agonist with low affinity for \(\alpha_1\), \(\beta_1\), \(\beta_2\), dopaminergic \(D_1\), \(D_2\), 5-HT\(_{2C}\), 5-HT\(_{1D}\) and GABA\(_A\) receptors, which is selective in functional assays of \(M_1\) receptor activation (Loudon et al. 1998). [\(^{14}\text{C}\)] labelled sabcomeline is highly brain penetrant in mice after oral or systemic administration and sabcomeline given intravenously (IV) induced hippocampal rhythmical slow wave activity (RSA) in anaesthetised rats (Loudon et al. 1998), which is indicative of postsynaptic \(M_1\) receptor activation (Barnes and Roberts 1991). At doses which evoked RSA of equivalent amplitudes, however, the changes in blood pressure and heart rate in the rat after sabcomeline (0.018 mg/kg IV) were 70% less than those seen after arecoline (0.1 mg/kg IV), suggesting that it possesses lower potency at \(M_2\) and \(M_3\) receptors (Loudon et al. 1998).

This study aimed to determine whether sabcomeline improves the performance of rats in a delayed alternation T-maze task. Delayed response procedures have previously been shown to be sensitive to cholinergic manipulations (Andrews et al. 1994; Davey et al. 1995). RS86 (2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide), a higher efficacy muscarinic agonist, and the cholinesterase inhibitor tacrine [THA (9-amino-1,2,3,4-tetrahydroaminoacridin)] were investigated for comparison. The averse properties of these compounds were studied using a conditioned taste aversion task.

**Materials and methods**

**Subjects**

Male Hooded Lister rats (OLAC) weighing approximately 200 g at the start of the experiment were used. Rats were housed in groups of six in a temperature controlled environment (20°C±1°C) and maintained on a 12-h light dark cycle (lights on 0700–1900 hours). For the maze task, rats were food deprived for 23 out of 24 h. Animals used in the conditioned taste aversion studies were allowed ad lib access to food throughout the study. Access to fluid was restricted to 0.8 ml twice daily. For the maze task only one food pellet was placed in each food well during this phase only one food pellet was placed in each food well. On trial one, the rat was placed in the start box for approximately 5–10 s, the guillotine door was raised and the rat was allowed to enter either of the goal arms. Having chosen an arm, the rat was confined to that arm until the food reward was consumed and was then returned to the start box. On the subsequent trials within the session, the animals were forced to choose alternating arms. The guillotine door of the previously visited arm was set to the closed position and the rat was forced to enter the previously unchosen arm. This alternating procedure was repeated until ten trials had been made or until 10 min had elapsed, whichever was the sooner.

During the final phase the rats were trained to alternate arm choices. On the first trial, each rat was placed in the start box and, upon release, was allowed a free choice of arms. Both arms were baited for the first trial. On selection of an arm, the door was closed and the rat was allowed to eat the food reward. The animal was then placed back in the start box and upon release was again allowed a free choice of arms. A correct choice was made if the rat entered the previously unvisited arm. Each rat was given a total of ten trials per day, up to a maximum running time of 10 min. Training continued until the group achieved a mean score of at least 80% correct choices for 3 consecutive days, which took approximately 12–15 days.

Following completion of training the experiment was placed under computer control (BBC Master micro-computer) using custom written software to control all events and time delays between trials. At this point, delays were introduced between each trial by retaining the animal in the start box for 0, 10, 20, 30 or 40 s. Each rat was given each delay according to a Latin square design. From this study, the minimum effective delay (20 s) was chosen (see Results) for further drug studies. Two separate dose-response studies were conducted. In the first, sabcomeline (SB-202026) was studied in a low dose range (0.001–0.03 mg/kg IP, \(n=29\)) and in the second, the dose range was extended (0.03–1.0 mg/kg IP, \(n=28\)). The third T-maze experiment was a dose-response study of RS86 (0.1–3.0 mg/kg IP, \(n=30\)) and the final experiment was a dose-response study of THA (0.1–3.0 mg/kg IP, \(n=30\)).

**Apparatus**

The T-maze was constructed from matt black perspex. The stem was 90 cm long with two arms (40 cm in length) projecting at right angles to form the “T”. The walls of the maze were 20 cm high and the apparatus was mounted on a mobile frame (overall height 1 m). At the end of each arm was cut a panel into which a small food well was placed. Food pellets were dropped into the well by a remotely controlled food dispenser (Camden Instruments Ltd), located behind the wall of the goal arm. A guillotine door at the base of the “T” stem formed a start box when closed, and two similar doors were placed at the entrance to each arm to confine the rat within an arm or to restrict access to that arm. Pairs of photocells were located 1 cm from the start box and 2 cm into each goal arm. These photocell beams allowed for complete automation and accurate timing of responses.

The apparatus was housed in a small room containing standard laboratory furniture, computer equipment and a rack for holding animal cages. On the wall immediately above the maze were two posters. All of these objects were designed to provide extramaze cues.

**Procedure**

Training was conducted 5 days a week for approximately 8 weeks, during which time the maze was operated manually. Habituation lasted for 5 days, during which each rat was placed in the maze for a period of 10 min each day. Both food wells were filled with approximately 12×45 mg food pellets (Camden Instruments Ltd) and pellets were also placed throughout the length of both arms and along the stem in order to encourage the rats to explore and enter the goal arms.

The second phase of training lasted 5 days. During this period, rats were forced to alternate their arm choices on ten daily trials, and during this phase only one food pellet was placed in each food well. On trial one, the rat was placed in the start box for approximately 5–10 s, the guillotine door was raised and the rat was allowed to enter either of the goal arms. Having chosen an arm, the rat was confined to that arm until the food reward was consumed and was then returned to the start box. On the subsequent trials within the session, the animals were forced to choose alternating arms. The guillotine door of the previously visited arm was set to the closed position and the rat was forced to enter the previously unchosen arm. This alternating procedure was repeated until ten trials had been made or until 10 min had elapsed, whichever was the sooner.

During the final phase the rats were trained to alternate arm choices. On the first trial, each rat was placed in the start box and, upon release, was allowed a free choice of arms. Both arms were baited for the first trial. On selection of an arm, the door was closed and the rat was allowed to eat the food reward. The animal was then placed back in the start box and upon release was again allowed a free choice of arms. A correct choice was made if the rat entered the previously unvisited arm. Each rat was given a total of ten trials per day, up to a maximum running time of 10 min. Training continued until the group achieved a mean score of at least 80% correct choices for 3 consecutive days, which took approximately 12–15 days.

Following completion of training the experiment was placed under computer control (BBC Master micro-computer) using custom written software to control all events and time delays between trials. At this point, delays were introduced between each trial by retaining the animal in the start box for 0, 10, 20, 30 or 40 s. Each rat was given each delay according to a Latin square design. From this study, the minimum effective delay (20 s) was chosen (see Results) for further drug studies. Two separate dose-response studies were conducted. In the first, sabcomeline (SB-202026) was studied in a low dose range (0.001–0.03 mg/kg IP, \(n=29\)) and in the second, the dose range was extended (0.03–1.0 mg/kg IP, \(n=28\)). The third T-maze experiment was a dose-response study of RS86 (0.1–3.0 mg/kg IP, \(n=30\)) and the final experiment was a dose-response study of THA (0.1–3.0 mg/kg IP, \(n=30\)).

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