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The profile of sabcomeline (SB-202026), a functionally selective M_1 receptor partial agonist, in the marmoset

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1 Sabcomeline (SB-202026, 0.03 mg kg^{-1}, p.o.), a potent and functionally selective M_1 receptor partial agonist, caused a statistically significant improvement in the performance of a visual object discrimination task by marmosets. No such improvement was seen after RS86 (0.1 mg kg^{-1}, p.o.).

2 Initial learning, which only required an association of object with reward and an appropriate response to be made, was not significantly affected. Reversal learning, which required both the extinction of the previously learned response and the acquisition of a new response strategy, was significantly improved after administration of sabcomeline (0.03 mg kg^{-1}, p.o.).

3 Sabcomeline (0.03 and 0.1 mg kg^{-1}, p.o.) had no significant effect on mean blood pressure measured for 2 h after administration in the conscious marmoset.

4 Sabcomeline (0.03 mg kg^{-1}, p.o.) caused none of the overt effects such as emesis or behaviours often seen after the administration of muscarinic agonists, e.g. face rubbing and licking.

5 This is the first study to demonstrate cognitive enhancement by a functionally selective M_1 receptor partial agonist in a normal (i.e. non-cognitively impaired) non-human primate and this effect was seen at a dose which did not cause side effects.

6 Perseverative behaviour and deficient acquisition of new information are seen in patients with Alzheimer's disease (AD). Therefore the data suggest that sabcomeline might be of therapeutic benefit in the treatment of AD.

Keywords: Learning and memory; sabcomeline; M_1 partial agonist; blood pressure; emesis

Introduction

Studies investigating compounds with pharmacological selectivity for muscarinic receptor subtypes, in which clone expression systems are used, have often failed to correlate with endogenous receptor studies due to variables such as cell line, receptor reserve, G protein density and tissue type (Eglen & Watson, 1996). As yet, unambiguously selective muscarinic M_1 receptor agonists have not been identified. However, compounds which demonstrate functional selectivity or partial selectivity for the muscarinic M_1 subtype, compared to other muscarinic receptor subtypes, have been shown to enhance cognitive performance in rodents, e.g. AF102B (FKS-508) (Nakahara *et al*., 1988), BIMC 182 (Cereda *et al*., 1994), CI-979 (M'Harzi *et al*., 1995), McN-A-343 (Ukai *et al*., 1995) and YM796 (Suzuki *et al*., 1995), and may therefore be of use in the symptomatic treatment of Alzheimer's disease (AD) in which there is a widespread degeneration of ascending cholinergic projections (Whitehouse *et al*., 1982; Lehericy *et al*., 1993).

Of the few muscarinic agonists for which clinical tolerance data have been obtained, all have caused cholinergic side effects to a varying degree (see Cutler & Sramek, 1995 for review). Xanomeline (LY246708 tartrate), a functionally selective M_1 agonist, caused diarrhoea, nausea and emesis in AD patients at 115 mg t.i.d (Fisher & Barak, 1994) and doses up to 75 mg t.i.d. caused blood pressure and heart rate changes in normal subjects and Alzheimer's patients (Medina *et al*., 1997). Also salivary amylase levels increased at higher doses of xanomeline which is indicative of M_3 activation (Sramek *et al*., 1995a,b) and suggests that M_1 selectivity may be dose-limited. CI-979 at doses of 2 mg and above evoked cholinergic symptoms and Parkinsonian behaviour which were dose limiting in a tolerability study on normal and Alzheimer's patients (Sramek *et al*., 1995). AF102B caused hypersalivation, vomiting, abdominal pain and diaphoresis after a single 50 mg dose in one study (Ohtani *et al*., 1990) but in another, 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). It is, therefore, essential in the identification of a successful treatment for AD that the dose of a compound which enhances cognition is clearly separated from that evoking side effects.

Sabcomeline (SB-202026) is the hydrochloride salt of (**R**-(Z)-(+)-α-(methoxyimino)-1-azabicyclo [2.2.2] octane-3-acetonitrile) (Figure 1, Bromidge *et al*., 1994). It is a potent and functionally selective M_1 partial agonist with low affinity for α_1-, β_1-, β_2-adrenoceptors dopamine D_1, D_2; 5-HT_{1C}, 5-HT_{1D} and $GABA_A$ receptors (Loudon *et al*., 1997). ^{14}C labelled sabcomeline administered p.o., s.c. or i.p. was highly brain penetrant in mice (Loudon *et al*., 1997). Sabcomeline (0.018 mg kg^{-1}, i.v.) induced hippocampal rhythmical slow wave activity (RSA) in the anaesthetized rat (Loudon *et al*., 1997), indicative of postsynaptic M_1 receptor activation (Barnes & Roberts, 1991). The RSA amplitude at this dose was equivalent to that produced by a 15 fold higher dose of arecoline. Cardiovascular changes are modulated peripherally by activation of M_2 receptors on the heart (Wilffert *et al*., 1983) and M_3 receptors on the vasculature (Clague *et al*., 1985). Changes in blood pressure and heart rate in the anaesthetized rat after sabcomeline (0.018 mg kg^{-1}, i.v.) were 70% less than those seen after arecoline (0.1 mg kg^{-1}, i.v.). Effects due to sabcomeline did not increase with increasing dose which is

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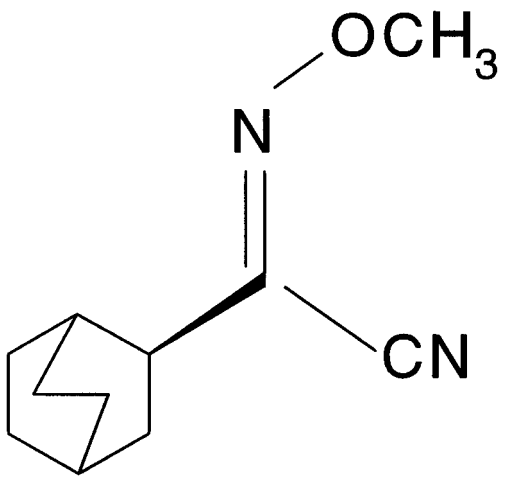

Figure 1 The structure of sabcomeline.

indicative of a partial agonist (Loudon *et al*., 1997). Thus, in contrast to its potency at muscarinic M_1 receptors sabcomeline evoked only minimal changes in heart rate and blood pressure, suggesting lower efficacy for the cardiovascular system than the CNS (Loudon *et al*., 1997).

Cognition enhancing effects have been observed after sabcomeline (0.03 mg kg^{-1}, i.p.) in both scopolamine-treated and normal rats. Sabcomeline reversed scopolamine- and delay-induced deficits in rat T-maze performance at doses approximately ten times lower than those inducing 'aversive' side effects in a conditioned taste aversion paradigm (Loudon *et al*., 1996).

This study aimed to determine whether a dose of sabcomeline, which failed to evoke blood pressure changes, a side effect typical of cholinomimetics, enhanced performance of a visual object discrimination task in the marmoset. RS86 (2-ethyl-8-methyl-2,8-diazaspiro-[4,5]-decan-1,3-dion hydrobromide), a higher efficacy muscarinic agonist, was also investigated for comparison. Sabcomeline is well tolerated in man (Kumar *et al*., 1996) and is currently undergoing Phase III clinical trials for the treatment of AD.

Methods

Animal husbandry

Adult common marmosets (*Callithrix jacchus*), 30 ± 1 months old and 370 ± 16 g in weight were used in these studies. Animals were housed in single sex pairs in cages containing a nesting box and items such as tree branches, ladders, cardboard tubes, mirrors and small toys for environmental enrichment. Music from a local radio station was played at a low level during the day. During blood pressure experiments and on rest days animals received a diet of egg sandwiches, a fruit selection of bananas, apples and pears, and a mash consisting of Farex baby food with a powdered form of Mazuri monkey chow. Animals were fed in the afternoon. During visual object discrimination testing animals received half the usual food allowance which was given after each session. Partial food deprivation was found to be essential for consistent and reliable performance of the task. The temperature in the holding rooms was kept at $25 \pm 1°C$ at a humidity of 55%. Rooms were illuminated between 07 h 00 min and 19 h 00 min.

Mean blood pressure

Animals and apparatus Sixteen marmosets (10 male, 6 female) were tested. None of the animals had been used previously in any study. An electronic blood pressure monitor, (W + W, model 8005), with a tail cuff and pressure sensor were used. The restraint was constructed from a clear perspex tube 250 mm in length and 85 mm in diameter, mounted on a perspex stand. The closed end of the tube was furnished with breathing holes.

Experimental procedure The marmoset was placed in the tube head first with its tail hanging out of the restraint. The animal was held in place by inserting a piece of perspex, with a cut out for the tail, at the base of the animal. The placement of this perspex piece could be varied to allow the animal to rest in a comfortable position before blood pressure recordings were taken via the tail cuff. Each animal was placed in the restraint for half an hour to habituate and blood pressure was measured every two minutes. The animal was then removed from the restraint, orally dosed with water and placed back into the restraint. The next blood pressure reading was taken 10 min after the last pre-dose reading and subsequently every 5 min for one hour. The animal was again removed from the restraint and dosed with either sabcomeline or water and returned to the restraint. The first blood pressure reading was taken 10 min after the previous reading and then every 5 min for 2 h. This protocol familiarized the animal with the dosing procedure and allowed an estimate to be made of the rate of recovery of blood pressure following handling and dosing.

In both studies animals were randomly assigned to either the drug or control group. In the first study eight males were given 0.03 mg kg^{-1} sabcomeline and four (3 females, 1 male) given water as controls.

After three weeks 16 animals, including the 12 tested previously, were used in the second study. Eight (3 females, 5 males) received 0.1 mg kg^{-1} sabcomeline and eight (3 females, 5 males) were given water.

Blood pressure measurements were taken over a single 4 h session for each drug dose during a weekday morning. On these days animals were fed their normal daily diet in the afternoon.

Visual object discrimination

Animals and apparatus Seven marmosets (6 female, 1 male) were tested. Animals were experimentally naïve and had only been briefly familiarized with testing equipment. None of the animals had been used in any other study. Animals were trained in a miniature Wisconsin General Testing apparatus to perform an object discrimination task. Each animal was transported from the main colony and tested in a box ($18 \times 24 \times 18$ cm) with bars on one side through which a food reward could be retrieved. Subjects were tested individually in a darkened room separated from the main colony. Local radio station music was played at low volume as in the holding room, to reduce distracting background noises.

Experimental procedure Preliminary training involved familiarizing the animals with the test apparatus. At the start of training a pair of previously unseen objects of similar size, e.g. a green ridged bottle top and a toy train whistle, were selected. One object was designated as the reward stimulus, the second object was not rewarded. Each object covered one of two identical wells located in a retractable perspex block which was positioned centrally within reach of the marmoset. The animal had to displace the rewarded object in order to retrieve the

reward, a piece of puffed rice. If the incorrect object was touched the trial was terminated by lowering the dividing partition which isolated the animal from the objects and the trial recorded as an error. A consistent interval of 10 s between trials was maintained during which the partition was lowered, the appropriate well was baited according to a pseudorandom schedule and objects were replaced over the wells. There was no corrective procedure and no time limit for trial completion. Each animal was trained once daily for a maximum of 30 trials per day until it successfully learned to displace the correct object and retrieve the reward to a criterion of 9 correct trials out of 10 successive trials. The previously unrewarded object than became the rewarded object. Discrimination learning continued with the newly rewarded object until criterion was achieved. Animals underwent preliminary training until a stable level of performance was attained i.e. no significant improvement in accuracy of responding occurred after reversing and re-reversing the rewarded objects five times. To ensure that the two groups in the drug studies were equal in mean performance, animals were divided according to their rate of learning the task during preliminary training.

Animals were dosed 30 min before each daily testing session of 30 trials on consecutive days until they attained the 9/10 correct criterion. For the first study subjects were given either sabcomeline (0.03 mg kg^{-1}) or an equivalent volume of water. Initial discrimination learning and reversal learning were tested with a novel pair of objects, set A, until criterion was reached. Only the initial discrimination task and the first reversal were tested, since no significant improvement had been observed beyond this stage during preliminary training (see Results). After an interval of 3 days the group previously given sabcomeline now became the control group and the group previously given water now became the drug group. Testing was repeated with a new pair of objects, set B. After two weeks marmosets were briefly retrained on the object discrimination task. They received either RS86 (0.1 mg kg^{-1}) or an equivalent volume of water. Object discrimination was tested as for sabcomeline but with novel object sets C and D. The time taken to perform a correct trial was measured from the moment the partition was raised to the instant the object was touched. Variation in the speed of performance could be indicative of changes in arousal or motivation.

Drugs

Sabcomeline (0.03 and 0.1 mg kg^{-1}) and RS86 (0.1 mg kg^{-1}) were dissolved in water (vehicle) and administered p.o. in a volume of 1 ml kg^{-1}.

Data analysis

Mean blood pressure Preliminary statistical analysis (RS1, BBN Research Systems) used a 2-way ANOVA to compare blood pressure at a given time point for each animal after drug and vehicle treatments. Each time point during the two hour period after drug or vehicle was analysed separately. This analysis compared the interaction between blood pressure and time for each treatment. If the interaction was not significant then an overall treatment effect was analysed by performing a 1-way ANOVA on the mean blood pressure over the two hour period for each animal after drug or vehicle.

Visual object discrimination Data for sabcomeline and RS86 were analysed independently (DesignExpert, Stat-Ease Inc.).

Three-way ANOVA was used to compare the levels of accuracy with the two object sets used for initial and reversal learning after drug or vehicle treatment. Significant interactions were identified by *t* tests.

Results

Mean blood pressure

Salivation or very mild emesis was observed in three of the eight animals which received 0.1 mg kg^{-1} sabcomeline. No such effect was seen in animals treated with 0.03 mg kg^{-1} sabcomeline.

Blood pressure measurements for all animals tested are presented as time course graphs (Figure 2). In the 0.03 mg kg^{-1} study, after both groups had received vehicle and had undergone a 40 min acclimatization period, there was a significant (*t* test, $P=0.018$) divergence in mean blood pressure between the two groups.

Inspection of the measurements from individual animals showed that 3, all in the drug group, exhibited a transient increase in blood pressure during the first half of the 40 min period in the first (0.03 mg kg^{-1}) study. In the second (0.1 mg kg^{-1}) study the same 3 animals showed no such increase in blood pressure. Conversely, one of the animals in the control group of the first experiment had noticeably lower

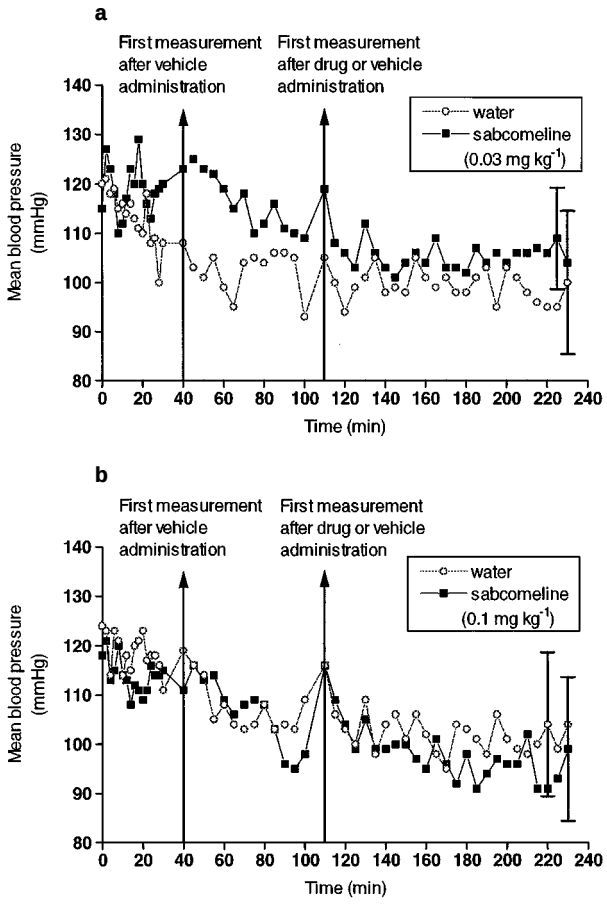

Figure 2 Mean blood pressure measurements for vehicle and drug-treated animals. Error bars represent a pooled s.e.mean between animals in each group. After 40 min acclimatization both groups were administered water p.o. After one hour ($t=110$ min) drug groups were orally dosed with (a) 0.03 mg kg^{-1} sabcomeline ($n=8$) or water ($n=4$) and (b) 0.1 mg kg^{-1} sabcomeline ($n=8$) or water ($n=8$).

blood pressure during the first half of the 40 min period than in the second study.

Mean blood pressure levels in both groups reconverged and did not differ significantly (t test, $P=0.1$) during the latter half of the acclimatization period. It is therefore, unlikely that the initial difference in mean blood pressure between the two groups influenced blood pressure measurements after drug or vehicle administration.

Blood pressure data were analysed for the 2 h period after drug or vehicle administration. There was no significant interaction between blood pressure and the time at which it was measured for each treatment group ($F(1.24)=0.54$, $P=0.96$), indicating that treatment effect followed a similar, parallel trend after drug or vehicle.

Mean blood pressure for each animal taken over the 2 h period after drug and vehicle was compared by one-way ANOVA. There was no significant difference in mean blood pressure of animals given vehicle and those given either 0.03 mg kg^{-1} ($F(1,10)=1.85$, $P=0.2$) or 0.1 mg kg^{-1} ($F(1,14)=0.9$, $P=0.36$) sabcomeline. There was no significant difference between mean blood pressure of male and female animals in the 0.03 mg kg^{-1} study (t test, $P=0.41$) or the 0.1 mg kg^{-1} study (t test, $P=0.88$).

Visual object discrimination

Preliminary training All animals successfully reached 9/10 correct criterion in initial and all reversal tasks. The rate at which criterion was reached in the initial task was significantly (independent 2-tail t test, $P<0.05$) more rapid than any of the reversal tasks R-1 to R-5 (Figure 3). Comparisons showed that there were no significant differences in the rates of attaining criterion between any of the reversal tasks. Neither did any animal perform the reversal tasks significantly better than any other.

These results suggest, therefore, that the marmosets had reached a stable level of performance at the end of the preliminary training and were unlikely to improve with further reversal training. Also, since no improvement occurred after the first reversal task compared to initial learning no additional testing seemed necessary. Therefore in subsequent experiments only the initial and first reversal tasks were performed.

Sabcomeline All subjects completed the experiment with no observable side effects. Table 1 shows the number of trials performed by each animal before nine correct trials out of ten successive trials were completed, i.e. a score of 26 denotes that 26 trials preceded the criterion run of 9 correct trials out of the next 10.

Initial learning was not significantly different when object set A or B was used. The object set used had a significant ($P=0.015$) effect on the rate of reversal learning. However, drug treatment greatly improved the rate of reversal learning

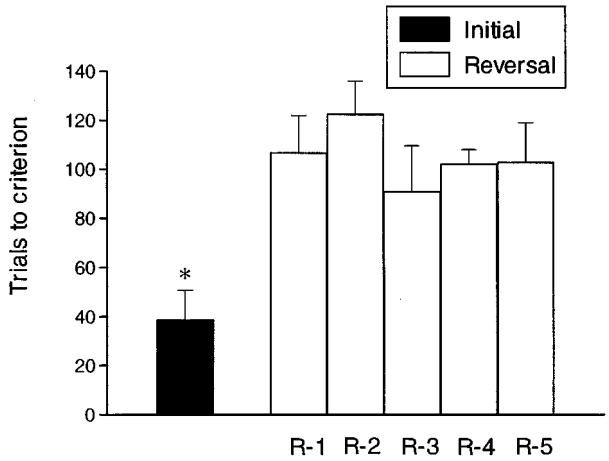

Figure 3 Trials (mean±s.e.mean) to criterion (9 correct in 10 successive trials) on a visual object discrimination task. Criterion was reached in significantly fewer trials in the initial task ($n=7$) than any of the reversal tasks ($n=7$), *$P<0.05$.

Table 1 Individual and mean scores of initial learning and reversal learning of visual object discrimination tasks

	Object set A		*Object set B*	
Study 1	*Initial*	*Reversal*	*Initial*	*Reversal*
Vehicle (water)	4	84	18	53
	8	105	11	65
	66	146	37	34
	42	118		
Mean±s.e.mean	30±14.7	113.3±13	22±7.8	50.7±9
Sabcomeline 0.03 mg kg^{-1}	26	30	12	21
	0	44	13	13
	0	17	4	56
			0	3
Mean±s.e.mean	8.7±8.7	30.3±7.8	7.3±3.2	23.3±11.5
	Object set C		*Object set D*	
Study 2	*Initial*	*Reversal*	*Initial*	*Reversal*
Vehicle (water)	11	86	88	52
	8	33	89	67
	53	69	17	90
Mean±s.e.mean	24±14.5	62.7±15.6	64.7±23.8	69.7±11.1
RS86 0.1 mg kg^{-1}	29	81	46	58
	35	85	55	54
	63	83	36	66
Mean±s.e.mean	42.3±10.5	83±1.2	45.7±5.5	59.3±3.5

Scores denote the number of trials to the start of the criterion run (9/10 correct).

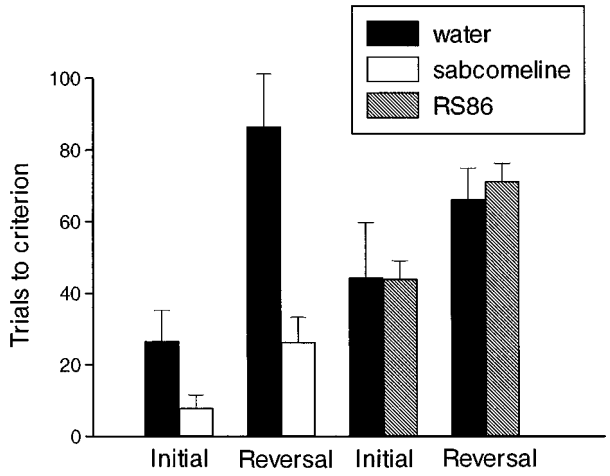

Figure 4 Trials (mean ± s.e.mean) to criterion (9 correct in 10 successive trials) on a visual object discrimination task. The left half of the figure shows initial and reversal learning after sabcomeline (0.03 mg kg^{-1}, p.o., $n=7$) or the equivalent volume of water. Sabcomeline had no significant effect on the rate of learning of the initial tasks compared to vehicle. Animals given sabcomeline made significantly ($P<0.005$) fewer errors during reversal task learning than vehicle-treated animals. Reversal learning following sabcomeline did not significantly differ from initial learning after drug or vehicle. The right half of the figure shows, in a separate study, initial and reversal learning after RS86 (0.1 mg kg^{-1}, p.o., $n=6$) or water. There was no significant difference in initial or reversal learning after drug or vehicle.

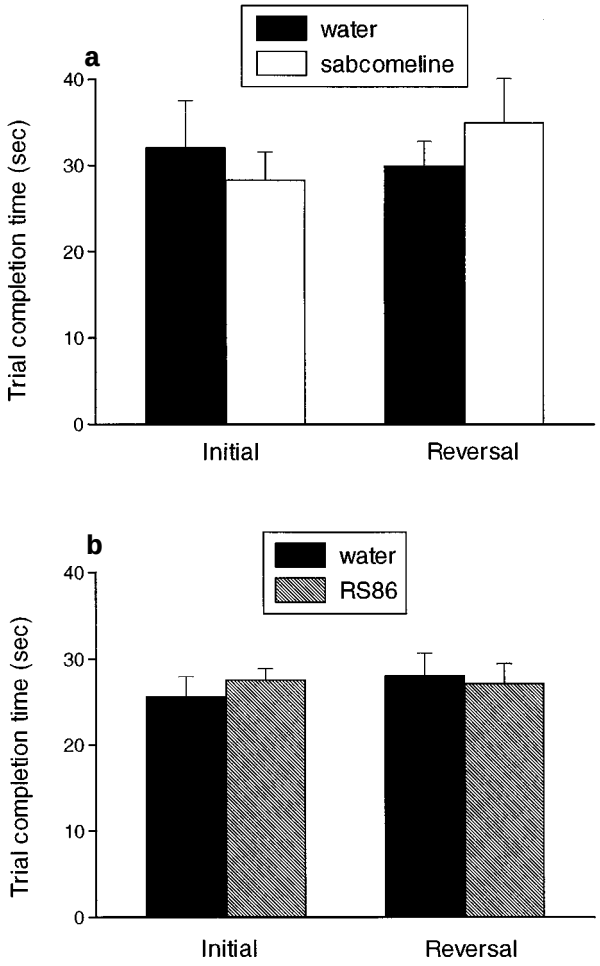

Figure 5 Time (mean ± s.e.mean) taken to perform a correct trial during initial and reversal tasks after (a) sabcomeline (0.03 mg kg^{-1} p.o.) or the equivalent volume of water (solid columns) and (b) RS86 (0.1 mg kg^{-1} p.o.) or water. Mean trial time did not significantly differ after sabcomeline and vehicle or after RS86 and vehicle.

with both object sets compared with vehicle. Data for object sets A and B were therefore combined. The rate of reversal learning after sabcomeline was significantly ($F(3,24)=12.67$, $P<0.005$) quicker than reversal learning after vehicle (Figure 4). Reversal learning after sabcomeline was not significantly different from the rate of learning of the initial task after either drug or vehicle.

The mean time taken to perform a correct trial did not significantly differ between drug and vehicle groups during performance of initial and reversal tasks (Figure 5), suggesting that motor function and general arousal levels were not altered after treatments.

RS86 One marmoset exhibited a single incidence of emesis following administration of RS86 and was immediately returned to the home cage, kept under observation and took no further part in the study. Scores for the remaining six animals were analysed in the same way as for sabcomeline.

The number of trials taken to reach criterion differed significantly ($F(1,4)=8.48$, $P=0.044$) between object set C and D (Table 1). The number of trials taken to reach criterion in the initial task was significantly ($P=0.028$) fewer than in the reversal task. There was no significant interaction between treatment and task ($P=0.79$) indicating that the effect of RS86 was similar in both initial and reversal tasks. There was no significant ($F(3,24)=2.59$, $P>0.05$) improvement in performance of either initial or reversal learning after administration of RS86 compared to vehicle (Figure 4). The mean time taken to peform a trial after drug or vehicle did not differ significantly (Figure 5).

Due to the different rates of learning between the two drug groups, subjects given sabcomeline received a maximum of only 2 consecutive daily doses before completing the task. A maximum of 6 doses of RS86 were required before task completion. One animal was dosed on 4 consecutive days when half the normal dietary allowance was given. There was no obvious weight reduction during this period.

Discussion

This study provides evidence for the first time of enhanced object discrimination learning by an orally administered, functionally selective muscarinic M_1 partial agonist, sabcomeline, in normal marmosets compared to control animals. At this cognition enhancing dose, 0.03 mg kg^{-1}, there was no evidence of changes in blood pressure or side effects related to autonomic cholinergic stimulation. The doses of sabcomeline were based on unpublished observations showing that 0.1 mg kg^{-1}, p.o., sabcomeline was the maximum tolerated dose evoking, in only some animals, mild emesis or related behaviours e.g. face rubbing and licking. Similarly, in an early efficacy study of patients with probable AD, sabcomeline was well tolerated with the lowest dose tested showing a side effect profile indistinguishable from placebo-treated subjects, whilst being as effective as higher doses on the ADAS-Cog scale (Kumar *et al*., 1996). The results presented here support evidence that functionally selective muscarinic M_1 receptor partial agonists are able to modulate learning and memory

(Nakahara *et al*., 1988; Cereda *et al*., 1994; M'Harzi *et al*., 1995; Ukai *et al*., 1995; Suzuki *et al*., 1995; Loudon *et al*., 1996).

In non-human primates studies supporting a role for the cholinergic system in learning and memory have often involved the imposition of a cognitive deficit e.g. by scopolamine (Bartus & Johnson, 1976; Ridley *et al*., 1984) or hemicholinium (Ridley *et al*., 1984). More recent investigation of the role of high (RS86) and low efficacy (AF-102B and L-689660) muscarinic receptor agonists in non-human primate cognition also used pharmacologically induced impairment (Rupniak *et al*., 1992). In that study assessment of a visuospatial memory task in scopolamine-impaired rhesus monkeys occurred after i.m. drug administration.

However, thermoregulation and emesis related side effects were monitored after p.o. administration in unimpaired squirrel monkeys. Reversal of learning deficits was only partial and remained significantly poorer than in unimpaired controls. Under such experimental conditions, where route of drug administration, species tested and behavioural state differ, it is extremely difficult to estimate the dose separation between potency to enhance learning and cholinergic side effects. In the present study there was no pharmacologically-induced cognitive deficit to overcome in order to show improved learning. All animals were dosed orally, in visual object discrimination and blood pressure experiments (and in the preliminary study in which emesis-related behaviours were observed) with drug or vehicle.

Consistency of these parameters allowed a direct comparison to be made between studies. The difference in performance levels in the preliminary training exercise showed that the reversal of discrimination tasks was significantly more difficult to acquire than the initial task. Serial reversal tasks were not significantly different from each other, indicating that a stable level of performance had been reached. In all marmosets sabcomeline elicited a clear-cut improvement in the rate of attaining 9/10 correct criterion in the reversal learning task compared with vehicle treated animals, reducing the number of trials required to a level close to that seen in initial learning. Furthermore, the mean time taken to complete a trial did not significantly differ after sabcomeline compared to vehicle, suggesting that arousal and motiviation levels, as well as motor function, were not unduly affected and did not impair task performance.

Marmosets with lesions of the basal forebrain, including the nucleus basalis of Meynert, an area which degenerates in AD (Whitehouse *et al*., 1982), had reduced choline acetyltransferase activity in anterior cortical regions (Roberts *et al*., 1990). These animals also exhibited a perseverative tendency during performance of a visual discrimination serial reversal task, continuing to choose the previously correct stimulus rather than the newly rewarded stimulus. It is therefore conceivable that, in the present study, the cognition enhancing effect of sabcomeline is due, in some part, to an increased ability to extinguish a perseverative response. Whereas, vehicle treated animals continue to respond to the previously rewarded object for a significantly longer period during the reversal task. In diseases such as dementia, schizophrenia and aphasia perseveration is an early marker of brain dysfunction (Freeman & Gathercole 1966; Allison & Hurwitz, 1967). Analysis of verbal descriptive discourse in AD patients, for example, showed that perseveration of words, phrases and ideas occurred significantly more frequently than in normal subjects (Bayles *et al*., 1985). A treatment which reduces perseverative behaviour could aid learning and acquisition of new information in such patients.

The dose of RS86 as based on data from an unpublished study in which 0.3 mg kg^{-1}, p.o., evoked severe emesis, diarrhoea and related side effects in all animals, whereas 0.1 mg kg^{-1} p.o. had a much lesser effect and did not affect all animals. These data are in agreement with another study in which RS86, at doses of 0.25 mg kg^{-1}, p.o., and above, evoked emesis in squirrel monkeys (Rupniak *et al*., 1992). There was no significant improvements in visual discrimination learning after RS86 compared with vehicle-treated animals. Results showed that there was an effect of object set on task performance which just reached statistical significance. In fact this result shows that with object set C attaining criterion in the reversal learning task took longer after RS86 than vehicle. However, with object set D, attaining criterion in the reversal learning task was faster after RS86 than vehicle. There was no evidence of a statistically robust improvement similar to that observed after sabcomeline. The inability of RS86 to improve performance accuracy at the dose tested in this study might be explained by its narrow therapeutic window, the separation between the dose required to enhance cognitive performance and that which evokes cholinergic side effects such as emesis, although a range of doses would have to be tested to confirm this. Such a lack of therapeutic separation is common with cholinoceptor agonists (Wettstein & Spiegel, 1984) and could, in this instance, be explained by the higher intrinsic efficacy and long lasting activity of RS86. Arecoline, another high efficacy muscarinic agonist, has also produced variable results when tested in AD patients. A miniority of patients showed no significant improvement at any dose which was effective in the majority (Raffaele *et al*., 1991a). Effective doses were sometimes task-dependent (Raffaele *et al*., 1996) or were independent of task but varied across subjects at a given dose (Raffaele *et al*., 1991b). In animal studies an inverted U-shaped dose-response curve has been observed after various cholinomimetics, suggesting that cognition is disrupted rather than being enhanced as the dose increases (Haroutunian *et al*., 1985). Any of these reasons could go some way to explaining the failure of RS86 to improve significantly task performance in the marmoset.

This study has shown that sabcomeline, a functionally selective muscarinic M_1 receptor partial agonist, significantly enhanced cognitive function in the marmoset. Compounds with this pharmacological profile might be of greater therapeutic value than muscarinic full agonists in the treatment of cognitive disorders, such as AD. Such a treatment might reduce the previously observed variability in cognitive enhancement and reduce the high number of AD patients who withdraw from studies as a result of dose-limiting side effects.

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References

ALLISON, R.S. & HURWITZ, L.J. (1967). On perseveration in aphasics. *Brain,* **90,** 429–448.

BARNES, J.C. & ROBERTS, F.F. (1991). Central effects of muscarinic agonists and antagonists on hippocampal theta rhythm and blood pressure in the anaesthetised rat. *Eur. J. Pharmacol.,* **195,** 233–240.

BARTUS, R.T. & JOHNSON, H.R. (1976). Short-term memory in the rhesus monkey: disruption from the anticholinergic scopolamine. *Pharmacol. Biochem. Behav.,* **5,** 39–46.

BAYLES, K.A., TOMOEDA, C.K., KASZNIAK, A.W., STERN, L.Z. & EAGANS, K.K. (1985). Verbal perseveration of dementia patients. *Brain Lang.,* **25,** 102–116.

BROMIDGE, S.M., CASSIDY, F., CLARK, M.S.G., EGGLESTON, D.S. & ORLEK, B.S. (1994). Synthesis and properties of [R-(Z)]-(+)-α-(1-azabicyclo [2.2.2] oct-3-yl)-α-(methoxyimino) acetonitrile, a novel functionally selective muscarinic partial agonist. *J. Chem. Soc. Chem. Commun.,* **18,** 2189–2190.

CEREDA, E., BRAMBILLA, A., EZHAYA, A., GAIDA, W., MEDLA, K., MONFERINI, E., PITSIKAS, N., SCHIAVI, G.B. & SAGRADA, A. (1994). Medicinal chemistry of muscarinic agonists for the treatment of dementia disorders. *Eur. J. Drug Metab. Pharmacokinet.,* **19,** 179–183.

CLAGUE, R.U., EGLEN, R.M., STRACHAN, A.C. & WHITING, R.L. (1985). Action of agonists and antagonists at muscarinic receptors present on ileum and atria *in vitro*. *Br. J. Pharmacol.,* **86,** 163–170.

CUTLER, N.R. & SRAMEK, J.J. (1995). Muscarinic M_1-receptor agonists: potential in the treatment of Alzheimer's disease. *CNS Drugs,* **3,** 467–481.

EGLEN, R.M. & WATSON, N. (1996). Selective muscarinic receptor agonists and antagonists. *Pharmacol. Toxicol.,* **78,** 59–68.

FISHER, A. & BARAK, D. (1994). Progress and persepectives in new muscarinic agonists. *Drug News Perspect.,* **7,** 453–464.

FISHER, A., HELDMAN, E., GURWITZ, D., HARING, R., KARTON, Y., MESHULAM, H., PITTEL, Z., MARCIANO, D., BRANDEIS, R., SADOT, E., BARG, Y., PINKAS-KRAMARSKI, R., VOGEL, Z., GINZBURG, I., TREVES, T.A., VERCHOVSKY, R., KLIMOWSKY, S. & KORCZYN, A.D. (1996). M_1 agonists for the treatment of Alzheimer's disease. Novel properties and clinical update. *Ann. New York Acad. Sci.,* **777,** 189–196.

FREEMAN, T. & GATHERCOLE, C.E. (1966). Perseveration – the clinical symptoms – in chronic schizophrenic and organic dementia. *Br. J. Psychiatry,* **112,** 27–32.

HAROUTUNIAN, V., BARNES, E. & DAVIS, K.L. (1985). Cholinergic modulation of memory in rats. *Psychopharmacology,* **87,** 266–271.

KUMAR, R., CEDAR, E., CLARK, M.S.G., LOUDON, J.M. & MCCAFFERTY, J.P.C. (1996). Muscarinic partial agonists in the symptomatic treatment of Alzheimer's disease. In *Alzheimer Disease: From Molecular Biology to Therapy,* ed. Becker R. & Giacobini E. pp. 299–303, Boston: Birkhauser.

LEHERICY, S., HIRSCH, E.C., CERVERA-PIEROT, P., HERSH, L.B., BAKCHINE, S., PIETTE, F., DUYCKAERTS, C., HAUW, J-J., JAVOY-AGID, F. & AGID, Y. (1993). Heterogeneity and selectivity of the degeneration of cholinergic neurons in the basal forebrain of patients with Alzheimer's disease. *J. Comp. Neurol.,* **330,** 15–31.

LOUDON, J.M., BROMIDGE, S.M., BROWN, F., CLARK, M.S.G., HATCHER, J.P., HAWKINS, J., RILEY, G.J., NOY, G. & ORLEK, B.S. (1997). SB-202026: A novel muscarinic partial agonist with functional selectivity for M_1 receptors. *J. Pharmacol. Exp. Ther.,* **283,** 1059–1068.

LOUDON, J.M., HATCHER, J.P., STOREY, V.J. & CLARK, M.S.G. (1996). SB-202026, a functionally selective M_1 partial agonist, enhances cognition at doses which do not induce side effects. *Neurobiol. Aging,* **17,** S30.

MEDINA, A., BODICK, N., GOLDBERGER, A.L., MACMAHON, M. & LIPSITZ, L.A. (1997). Effects of central muscarinic-1 receptor stimulation on blood pressure regulation. *Hypertension,* **29,** 828–834.

M'HARZI, M., PALOU, A.M., OBERLANDER, C. & BARZAGHI, F. (1995). Antagonism of scopolamine-induced memory impairments in rats by the muscarinic agonist RU 35, 926 (CI-979). *Pharmacol. Biochem. Behav.,* **51,** 119–124.

NAKAHARA, N., IGA, Y., MIZOBE, F. & KAWANISHI, G. (1988). Amelioration of experimental amnesia (passive avoidance failure) in rodents by the selective M_1 agonist AF102B. *Jpn. J. Pharmacol.,* **48,** 502–506.

OHTANI, Y., MURASAKI, M., ISHII, Y., ISHIGOOKA, J. & MUIRA, S. (1990). Phase I study of FKS-508 single and multiple dose studies. *Eur. J. Pharmacol.,* **183,** 1038.

RAFFAELE, K.C., ASTHANA, S., BERARDI, A., HAXBY, J.V., MORRIS, P.P., SCHAPIRO, M.B. & SONCRANT, T.T. (1996). Differential response to the cholinergic agonist arecoline among different cognitive modalities in Alzheimer's disease. *Neuropsychopharmacology,* **15,** 163–170.

RAFFAELE, K.C., BERARDI, A., ASTHANA, S., MORRIS, P., HAXBY, J.V. & SONCRANT, T.T. (1991a). Effects of long term continuous infusion of the muscarinic cholinergic agonist arecoline on verbal memory in dementia of the Alzheimer type. *Psychopharmacol. Bull.,* **27,** 315–319.

RAFFAELE, K.C., BERARDI, A., MORRIS, P.P., ASTHANA, S., HAXBY, J.V., SCHAPIRO, M.B., RAPOPORT, S.I. & SONCRANT, T.T. (1991b). Effects of acute infusion of the muscarinic cholinergic agonist arecoline on verbal memory and visuo-spatial function in dementia of the Alzheimer type. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **15,** 643–648.

RIDLEY, R.M., BARRATT, N.G. & BAKER, H.F. (1984). Cholinergic learning deficits in the marmoset produced by scopolamine and I.C.V. hemicholinium. *Psychopharmacology,* **83,** 340–345.

ROBERTS, A.C., ROBBINS, T.W., EVERITT, B.J., JONES, G.H., SIRKIA, T.E., WILKINSON, J. & PAGE, K. (1990). The effects of excitotoxic lesions of the basal forebrain on the acquisition, retention and serial reversal of visual discriminations in marmosets. *Neuroscience,* **34,** 311–329.

RUPNIAK, N.M.J., TYE, S.J. & IVERSEN, S.D. (1992). Comparison of the effects of selective and nonselective muscarinic agonists on cognition and thermoregulation in primates. *J. Neurol. Sci.,* **110,** 222–227.

SRAMEK, J.J., CUTLER, N.R., HURLEY, D.J. & SEIFERT, R.D. (1995a). The utility of salivary amylase as an evaluation of M_3 muscarinic agonist activity in Alzheimer's disease. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **19,** 85–91.

SRAMEK, J.J., SEDMAN, A.J., REECE, P.A., HOURANI, J., BOCKBRADER, H. & CUTLER, N.R. (1995b). Safety and tolerability of CI-979 in patients with Alzheimer's disease. *Life Sci.,* **57,** 503–510.

SUZUKI, M., YAMAGUCHI, T., OZAWA, Y., IWAI, A. & YAMAMOTO, M. (1995). Effects of YM796, a novel muscarinic agonist, on the impairment of passive avoidance response in senescence-accelerated mice. *Pharmacol. Biochem. Behav.,* **51,** 623–626.

UKAI, M., SHINKAI, N. & KAMEYAMA, T. (1995). Cholinergic receptor agonists inhibit pirenzipine-induced dysfunction of spontaneous alternation performance in the mouse. *Gen. Pharmacol.,* **26,** 1529–1532.

WETTSTEIN, A. & SPIEGEL, R. (1984). Clinical trials with the cholinergic drug RS86 in Alzheimer's disease (AD) and senile dementia of the Alzheimer type (SDAT). *Psychopharmacology,* **84,** 572–573.

WHITEHOUSE, P.J., PRICE, D.L., STRUBLE, R.G., CLARK, A.W., COYLE, J.T. & DELONG, M.R. (1982). Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science,* **215,** 1237–1239.

WILFFERT, B., DAVIDESKO, D., DE JONGE, A., THOOLEN, M.J.M.C., TIMMERMANS, P.B.M.W.M. & VAN ZWEITEN, P.A. (1983). Role of ganglionic M-1 and M-2 receptors in the neuronal control of the cardiovascular system of the normotensive rats as determined with pilocarpine. *Eur. J. Pharmacol.,* **95,** 109–116.

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