R-plasmid was always transferred as a single unit irrespective of the selective agent i.e. ampicillin, trimethoprim, sulphonamide, tetracycline or chloramphenical using nalidixic acid as the counter-selecting agent (Table 1). However, transconjugants obtained using J62-2, J53-2 and BN008 as recipients independently exhibited two types of resistance phenotype, i.e. types 6 and 7 using rifampicin as the counter-selective agent (Table 1). Type 6 and 7 transconjugants were further mated with recipients J62-1 and CA6. The transconjugants so obtained exhibited only the resistance phenotypes of the respective donors.

These results indicate that there could be two R-plasmids mediating resistance to these antibiotics, one R-plasmid mediating trimethoprim, sulphonamide and ampicillin resistance, and the other mediating ampicillin; tetracycline, chloramphenicol and sulphonamide resistance. This suggests that there were two different ampicillin genes and two different sulphonamide genes in the original isolate and the two R-plasmids were only separated on transfer during counterselection with rifampicin.

This is an unusual pattern of antibiotic resistance and also an unusual R-plasmid segregation by rifampicin. A similar result was reported by Willis & Smith (1978) which showed that it was the drug selection used which governed the types of R-plasmid obtained. These results, on the other hand, suggest that it is the strain of recipient used which governs the type of R-plasmid obtained. It could be speculated therefore that the use of rifampicin as a counterselective agent can result in the segregation of multiple R-plasmids often transferred as a single unit when other recipients are used.

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Central administration of the muscarinic receptor subtype – selective antagonist pirenzepine selectively impairs passive avoidance learning in the mouse

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There is now good evidence that muscarinic receptors in different parts of the body are not identical. Using radioligand binding techniques, Hammer et al (1980) showed that the anti-muscarinic drug pirenzepine had a greater affinity for receptors in glandular tissue than for muscarinic receptors in heart and smooth muscle. Pirenzepine also had higher affinity for muscarinic sites in the hippocampus and cortex than for sites in the medulla.

Activation of brain muscarinic receptors influences many processes, including motor function, body temperature regulation and pain sensation (see Karczmar 1977 for review). Additionally, there is evidence that cholinergic systems in the c.n.s. influence learning and memory. Thus, it has long been known that centrally-acting anticholinergics can disrupt learning of avoidance behaviour in rodents and interfere with memory functions in man (see review by Squire & Davis 1981). It is important to investigate the possible involvement of subtypes of the muscarinic receptor in these central processes. Should different subtypes be concerned with different functions, it would be predicted that subtype-selective compounds (such as pirenzepine) should influence some functions without affecting others.

We have therefore examined the effects of pirenzepine, given interacerebroventricularly, on a number of central effects mediated by muscarinic receptors and have found that pirenzepine impairs learning of a passive avoidance task at doses approximately fifty times lower than the doses which antagonize oxotremorine-induced tremor, hypothermia and antinociception.

Method

Mice (male Charles River CD-1, 20–24 g) were trained in groups of 20 in a one-trial step-through passive avoidance procedure using a two-compartment apparatus similar to that described by Jarvik & Kopp (1967). On being placed in the light compartment, the mouse was kept from entering the dark compartment for a familiarization period of 10 s, after which the intervening partition door was raised allowing the animal to enter the dark section. On entry, the animal was given

* Correspondence

an unavoidable footshock (0·5mA for 1s). It was then returned to its home cage until retest 24h. later, when it was placed in the apparatus as before. Latency to enter the dark compartment was recorded. Other anti-muscarinic actions of the drugs under test were assessed in separate experiments, in groups of at least ten mice, by measuring antagonism of effects seen 20 min following injection of oxotremorine (0·5 mg^{-1} kg i.p.). The effects measured were hypothermia, tremor and antinociception (using a tail-clip method). Intracerebroventricular (i.c.v.) injections were made into the third ventricle by the method of Haley & McCormick (1957). Accuracy of injections was checked in separate experiments by dye injection. It was found that dye penetrated the ventricles in 85% of animals. Drugs for i.c.v. injections were made up in Krebs-Henseleit solution and injected in a volume of 5 µl.

Results

Pirenzepine was found to impair passive avoidance learning when given i.c.v. 20 min pre-training. In a typical experiment, vehicle-injected animals had a median entry latency on retest of 61 s, showing highly significant learning compared to unshocked controls (median latency = 7·5 s). The median latencies in pirenzepine-treated animals were 79·5, 11, 27 and 25·5 seconds with doses of 0·03, 0·1, 0·3 and 1 µg per mouse respectively. These values were significantly lower than controls ($P < 0·05$, Mann-Whitney 'U'-test, except in the case of the lowest dose tested (0·03 µg). The effectiveness of pirenzepine in antagonizing the hypothermia, tremor, and antinociception produced by oxotremorine was considerably less (Table 1). ED50 values against these muscarinic effects ranged from 4·6 to 5·8 µg per mouse. Thus, pirenzepine was about 50 times more effective in producing impaired passive avoidance learning than in antagonizing other effects resulting from central muscarinic receptor activation. By contrast, the non-selective muscarinic antagonist, *N*-methyl atropine (also given i.c.v. 20 min pre-training) did not impair passive avoidance learning at doses of 0·03–1 µg, but was effective at 3 and 10 µg. The doses of methyl atropine effective in the anti-oxotremorine test were 1·2–2·0 µg (Table 1).

Conclusion

In conclusion, we have shown that the selective anti-muscarinic drug pirenzepine appears to be very potent in impairing learning of an avoidance; much higher doses are required to antagonize other central muscarinic effects. By contrast, a non-selective antimuscarinic (*N*-methyl atropine) was active against passive avoidance learning and oxotremorine-induced hypothermia, tremor and antinociception at similar doses. The implication from these preliminary findings is, therefore, that the muscarinic receptors thought to be involved in learning and memory processes are of a different type to the receptors involved in other central muscarinic effects. However, since possible non-specific drug effects in the passive avoidance test have not been excluded in these experiments, this conclusion is, at best, tentative.

Table 1. A comparison of the minimum doses of pirenzepine and methyl atropine effective in impairing learning of a one-trial passive avoidance procedure, with doses of the drugs required to reduce the effects of a standard dose of oxotremorine by 50%.

	Pirenzepine	Methyl atropine
A		
Passive avoidance—MED	0.1	3
B		
Anti-oxotremorine test 'ED50'		
hypothermia	4·8 (3·2–6·4)	2·0 (0·9–3·1)
tremor	5·8 (3·1–9·7)	1·3 (0·9–1·8)
antinociception	4·6 (2·8–7·4)	1·2 (0·9–1·7)

A. Minimum effective dose producing impaired passive avoidance learning (µg per mouse i.v.c.). Retest latencies were significantly less than control latencies ($P < 0·001$).

There was no significant difference in median retest latencies between the three effective pirenzepine doses (0·1 µg – 1 µg). As the drug effect was not quantitatively dose-related over this range, ED50, values could not be calculated. Therefore, the efficacy of pirenzepine in this test has been expressed as the smallest dose which was effective in impairing passive avoidance learning.

B. Dose required to reduce the effect of oxotremorine (0·5 mg^{-1} kg i.p.) by 50% (µg per mouse i.c.v.).

Figures in brackets represent 95% confidence limits of the median doses shown. Experiments were carried out using 10–20 mice in each dose group.

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