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Segregation of R-plasmids in *Escherichia coli* K-12 rifampicin-resistant mutants

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R-plasmids mediating resistance to rifampicin have so far not been reported. Chromosomal rifampicin resistance has been encountered by exposing sensitive bacteria to inhibitory concentrations of rifampicin in the laboratory. This is often used as a counter-selection during the selection of R-plasmid resistance phenotypes in bacterial mating experiments.

An *Escherichia coli* possessing an R-plasmid, termed BN171, was isolated from a patient with a chronic urinary tract infection which had failed to respond to therapy with β-lactamase-stable penicillins and cotrimoxazole. BN171 was transferred to a laboratory strain of *E. coli* J62-1 (*pro*; *lac*; *trp*; *his*; with chromosomal nalidixic acid resistance, Coetzee et al 1972), using trimethoprim and nalidixic acid as the selective and counterselective agents, respectively. Resistance to ampicillin, tetracycline, sulphonamide, chloramphenicol and trimethoprim was exhibited in all the transconjugants. Transfer of BN171 to an isogenic recipient J62-2 (with chromosomal rifampicin-resistance, Coetzee et al 1972) produced transconjugants not possessing the complete spectrum of resistance phenotypes as was expressed in the J62-1 recipient (Table 1). Transfer of BN171 from J61-1 (BN171) to another *E. coli* J53-2 as recipient (*pro*; *met*, with chromosomal rifampicin-resistance, Coetzee et al 1972) also produced transconjugants not possessing the complete resistance phenotypes as was expressed in the J62-1 recipient. Such transconjugants obtained from the J53-2 recipient had resistance spectra similar to the transconjugants that were obtained using the J62-2 recipient. Transfer of BN171 from J62-1 (BN171) to another laboratory *E. coli K-12* recipient strain CA6 (*pro*; *asn*; *lac*, with chromosomal streptomycin-resistance), produced transconjugants exhibiting all the resistance phenotypes. Further transfer of BN171 from J62-1 (BN171) to BN008 (a rifampicin-resistant mutant of *E. coli* K-12 W3110, again exhibited transconjugants with resistance spectra similar to the transconjugants that had been obtained using the J62-2 and J53-2 recepients.

The presence of all the five resistance phenotypes in transconjugants using strains J62-1 and CA6 as recipients suggested that the R-plasmid conferring resistance to the five antibiotics was a single unit. The

Table 1. Resistance spectra of transconjugants obtained on transfer of R-plasmid BN 171 to nalidixic acid-resistant and rifampicin resistant mutants used as recipients.

Recipient	Counter-selective agent	Selective agent	Type	Resistance pattern					Frequency of transfer per donor
				Ap	Tp	Su	Cm	Tc	
J62-1	Nal	Ap	1	+	+	+	+	+	$2{\cdot}1 \times 10^{-5}$
,,	Nal	Tp	2	+	+	+	+	+	2×10^{-5}
,,	Nal	Su	3	+	+	+	+	+	$2{\cdot}2 \times 10^{-5}$
,,	Nal	Cm	4	+	+	+	+	+	$2{\cdot}1 \times 10^{-5}$
,,	Nal	Tc	5	+	+	+	+	+	$1{\cdot}8 \times 10^{-5}$
J62-2	Rif	Ap	6	+	−	+	+	+	$2{\cdot}5 \times 10^{-5}$
,,	Rif	Ap	7	+	+	+	−	−	$1{\cdot}1 \times 10^{-6}$
,,	Rif	Tp	8	+	+	+	−	−	$1{\cdot}2 \times 10^{-6}$
,,	Rif	Su	9	+	+	+	−	−	$1{\cdot}4 \times 10^{-6}$
,,	Rif	Su	10	+	−	+	+	+	$2{\cdot}1 \times 10^{-5}$
,,	Rif	Cm	11	+	−	+	+	+	$2{\cdot}2 \times 10^{-5}$
,,	Rif	Tc	12	+	−	+	+	+	$1{\cdot}4 \times 10^{-5}$
J53-2	Rif	Ap	13	+	−	+	+	+	$2{\cdot}4 \times 10^{-5}$
,,	Rif	Ap	14	+	+	+	−	−	$1{\cdot}5 \times 10^{-6}$
,,	Rif	Tp	15	+	+	+	−	−	$1{\cdot}2 \times 10^{-6}$
,,	Rif	Su	16	+	+	+	−	−	$1{\cdot}8 \times 10^{-6}$
,,	Rif	Su	17	+	−	+	+	+	2×10^{-5}
,,	Rif	Cm	18	+	−	+	+	+	$2{\cdot}2 \times 10^{-5}$
,,	Rif	Tc	19	+	−	+	+	+	$1{\cdot}8 \times 10^{-5}$

Ap = ampicillin, Tp = trimethoprim, Su = sulphonamide, Cm = chloramphenicol, Tc = tetracycline.
+ resistance found in transconjugant.
− resistance absent from transconjugant.

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

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