enzyme upfield shifts of the aromatic proton resonances of the ligand were observed. From the dependence of the shifts on ligand concentration both the affinity constant and the shift in the bound state could be determined. In the bound state the upfield shift is 0.41 and 0.58 ppm for the ortho- and meta-protons respectively; the addition of the coenzyme NADPH increases the affinity by a factor of 3.4 but the bound shifts are decreased. *p*-Nitrobenzoyl-L-glutamate (L-PNBG) shows similar shifts although the affinity is lower. On the other hand the protons of *p*-aminobenzoyl-D-glutamate (D-PABG) showed no shift on binding. In this case the binding constant was determined by competition with L-PABG or L-PNBG.

The presence of such large chemical shifts in the L-isomers on binding are probably due to ring current effects from aromatic amino acids in the immediate neighbourhood of the site at which the benzoyl group is bound. The benzoyl group in D-PABG must therefore be bound in a very different environment. Nevertheless the binding of both D- and L-PABG is abolished by methotrexate.

The spectrum of the enzyme shows similar changes in the resonance of three of the six histidines and also in the aromatic and methyl regions of the spectrum, when the ligands bind.

	without NADPH			*with NADPH*		
		Δ (ppm)			*Δ (ppm)*	
	K(10^3 M^{-1})	*ortho*	*meta*	*K(10^3 M^{-1})*	*ortho*	*meta*
L-PABG	1.05 ± 0.15	0.41 ± 0.02	0.58 ± 0.02	3.57 ± 0.35	0.30 ± 0.02	0.35 ± 0.02
L-PNBG	0.47 ± 0.06	0.36 ± 0.02	0.67 ± 0.02	1.51 ± 0.02	0.31 ± 0.02	0.45 ± 0.02
D-PABG	0.34 ± 0.08	< 0.05	< 0.05	1.4 ± 0.3	< 0.05	< 0.05

Two populations of acetylcholine receptors in guinea-pig ileum

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Propylbenzilylcholine mustard (PrBCM) is a potent alkylating muscarinic antagonist in smooth muscle and brain which can be labelled at high specific activity with tritium. The reaction of [^{3}H]-PrBCM with the receptor in microsomes prepared from homogenates of the longitudinal muscle of the guinea-pig ileum which probably contain myenteric plexus, can be antagonized by a variety of reversible antagonists. The affinity constants of these substances for the receptor can be estimated from the reduction in the reaction rate of alkylation by [^{3}H]-PrBCM. The values obtained with substances such as atropine agree satisfactorily with those reported from experiments in which the dose ratio of an antagonist is obtained in intact muscle strips. Furthermore the concentration-inhibition relationship is accurately described by a simple mass equation and when plotted as a Hill plot gives a slope of 1.0.

Agonists, such as acetylcholine, also inhibit alkylation by [^{3}H]-PrBCM, but in this case the concentration-inhibition curve is a poor fit to a mass equation and the slope of the Hill plot is 0.5-0.6. A Scatchard plot suggests the presence of two populations of binding sites for acetylcholine with affinities of 1.8×10^8 M^{-1} and 1.6×10^6 M^{-1} present in roughly equal amounts. The total receptor concentration revealed by acetylcholine inhibition is identical to that revealed by atropine.

The characteristics of these two receptor pools will be discussed.