a binding capacity of 1.2 fmole/mg (0.8-2.0 fmole/mg). The value of M/K was 0.14 μl/mg (0.12 to 0.18 μl/mg), i.e. there was a 2.8-fold reduction of TTX binding after denervation. Our results suggest that this was due to a reduction in binding capacity (M), rather than a decrease in affinity (increase in K). This is compatible with electrophysiological experiments in which it was found that the maximum rate of rise of the action potential became partially resistant to TTX after denervation (as found by Redfern & Thesleff, 1971). The maximum rate of rise in denervated muscle fell initially over the same range of TTX concentrations as for innervated muscle but when it had fallen by a factor of about 2, even high TTX concentrations produced little further reduction.

Detubulation with glycerol did not alter the TTX sensitivity of normal or denervated muscle.

These results suggest that denervated muscle possesses two kinds of sodium channel, one normally sensitive and the other resistant to TTX, but that these cannot be identified with channels in the transverse tubules and surface membrane respectively.

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Structure-activity relationships in the sulphonamide-carbonic anhydrase systems

A.S.V. BURGEN & R.W. KING*

National Institute for Medical Research, Mill Hill, London, NW7 1AA

In an attempt to elucidate the relationship between binding properties and chemical structure we have examined the reactions of several homologous series of sulphonamide inhibitors with Human Carbonic Anhydrase isoenzyme C. Using equilibrium binding and stopped flow fluorescence methods we have shown that a consistent pattern of changes in affinity constant and dissociation rate constant exists for all of the different series of homologous *para*-substituted benzene sulphonamides we have examined. An observed increase in affinity constant as a series is ascended, at least as far as C_5, is due both to an increase in association rate constant and a decrease in dissociation rate constant, though the relative contribution of these varies from series to series. Increasing affinity with increasing chain length also correlates with the increase in octanol-water partition coefficient.

We have also examined the effect of varying the ring position of an homologous series of esters of sulphamoyl benzoic acid. Substantial decreases in affinity constant accompany the change from para to meta substitution and also from meta to ortho substitution.

NMR studies of the binding of substrate analogues to L. casei dihydrofolate reductase

A.S.V. BURGEN*, J. DANN, J. FEENEY, G.C.K. ROBERTS & V. YUFEROV[1]

National Institute for Medical Research, Mill Hill, London, NW7 1AA

Dihydrofolate reductase is the target enzyme for a number of important chemotherapeutic agents. We have prepared pure enzyme from L. casei MTX/R (a methotrexate-resistant strain) and have studied the binding of substrate analogues by NMR spectroscopy. For the binding studies the enzyme was dissolved in 0.05 M K phosphate, 0.5 M KCl, pH (meter reading) 6.5 in D_2O at a concentration of 0.8-1.4 mM. Aliquots of concentrated ligand solution were added with a Hamilton syringe. The [^{1}H]-NMR spectra were obtained at 100 MHz using a Varian XL-100-15 spectrometer in the Fourier transform mode; 500 transients were accumulated. The probe temperature was $20 \pm 1°C$.

On addition of *p*-aminobenzoyl-L-glutamate (L-PABG), a fragment of the substrate, to the

[1] *Present address: Institute of Virology, Moscow, USSR.*

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

A.S.V. BURGEN & C.R. HILEY*

Division of Molecular Pharmacology, National Institute for Medical Research, London NW7 1AA

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.