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

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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

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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

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