of $1 + (x/K)$, where x is the concentration, and K the equilibrium dissociation constant, of the inhibitor.

To avoid diffusion limitation of the rate of BuTX binding homogenates of rat diaphragm (denervated 13-15 days previously) were used. Binding in intact muscle was found to be much slower, and, as expected, the degree of inhibition by TC much less.

The initial rate of BuTX binding (measured after 7 min incubation with [^{125}I]-BuTX) could be depressed to 10% or less of its control value by sufficiently high concentrations of TC, hexamethonium (C6), decamethonium (C10), carbachol (CCh) and suxamethonium (SCh). After subtraction of this small uptake of the BuTX which cannot be inhibited by (+)-tubocurarine, the initial rate was found to be related to inhibitor concentration as predicted above. This method gave equilibrium constants of 0.3 μM for TC, 175 μM for C6, 2.0 μM for C10, and 1.3 μM for SCh. The value for TC is near to the value (0.5 μM) calculated from the inhibition of CCh-induced depolarizations in denervated muscle fibres.

In other experiments the BuTX binding was followed nearly to equilibrium over 3 hours. The rate was measured in the presence of high concentrations of TC (60 μM) and CCh (800 μM) to obtain the uptake of the BuTX which cannot be inhibited. The latter at 3 h, was about 20% of the total uptake. After subtraction of this, the remaining uptake was approximately exponential, the rate constants indicating equilibrium constants of 0.33 μM for TC and 4 μM for CCh.

These experiments suggest that the finding that BuTX binding to the endplates of normal rodent muscle can be reduced to only about half the control value by high concentrations of TC (Berg *et al.*, 1972; Porter *et al.*, 1973) may not mean that only half the BuTX binding sites are ACh receptors. A reduced degree of inhibition of BuTX binding would be expected if measurements were made at a fixed time that was not short (compared with the rate constant for binding), or if the rate of BuTX binding were diffusion controlled.

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Tetrodotoxin binding to innervated and denervated rat diaphragm homogenates

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The binding of tritium labelled tetrodotoxin ([^{3}H]-TTX) to intact innervated rat diaphragm was reported by Colquhoun, Rang & Ritchie (1973). We have now measured the binding of [^{3}H]-TTX to denervated muscle, which is known to become partly resistant to the action of TTX (Redfern & Thesleff, 1971; Harris & Thesleff, 1971). Muscle homogenates were used, rather than intact muscle; this improved the precision of the binding measurements.

The homogenate, washed with 0.6 M KCl and resuspended in buffered saline, was incubated for 1-4 h at 20°C with [^{3}H]-TTX (Colquhoun, Henderson & Ritchie, 1972) followed by centrifugation at 10^5 *g*. The unbound [^{3}H]-TTX trapped in the pellet was measured by means of [^{14}C]-mannitol.

As before, the results could be fitted by the sum of a hyperbolic saturable component and a linear component. Pooling the results from intact and homogenized normal (innervated) diaphragm indicated a saturable component with an equilibrium constant of $K = 6.1$ nM (95% interval 4.7-7.8 nM), and a binding capacity of $M = 2.5$ fmole/mg wet weight (95% interval 2.1-2.8 fmole/mg). The value of M/K, which is more precisely determined than either M or K separately, was 0.40 μl/mg (0.36-0.46 μl/mg). The saturable component was abolished in the presence of 100 nM saxitoxin. In diaphragm muscle which had been denervated 5-14 days previously, the binding of [^{3}H]-TTX was reduced. Measurements on intact denervated muscle gave $M/K = 0.13$ μl/mg, but were not precise enough for M and K to be separately determined. Homogenized denervated muscle showed a saturable component of [^{3}H]-TTX binding with $K = 8.5$ nM (4.3 to 15.3 nM), little different from the value in innervated muscle, and

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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