The uptake of weak acids and bases into isolated rat superior cervical ganglia in relation to intracellular pH

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Under conditions where direct measurement is not feasible, intracellular pH (pH_i) may be estimated from the relative concentration of a weak acid or base in the intra- and extra-cellular fluids. Since its introduction by Waddell & Butler (1959), the weak acid 5,5-dimethyl-2,4-oxazolidinedione (DMO) has become the most frequently used marker for cell pH.

Previous measurements of [^{14}C]-DMO distribution in isolated sympathetic ganglia suggested a pH_i of 7.33 at an extra-cellular pH of 7.37 (Brown & Halliwell, 1972). However, the weak base nicotine (measured under conditions where cell depolarization was averted) accumulated in the ganglion to an extent requiring a much more acid environment (pH_i 6.5-6.6). To explain this, a hypothesis of different pH compartments within the cell was advanced, such that a weak base would distribute in accordance with the pH of the most acid compartment and vice versa for a weak acid; on this basis it was suggested that nicotine distribution might reflect the pH of the largest compartment (the cytoplasm) with greater accuracy than a weak acid.

In an attempt to further test this view—and to exclude possible peculiarities in the distribution of nicotine— the uptake of a number of weak bases and acids into isolated rat superior cervical ganglia, incubated in Krebs' solution (pH 7.4) at 25° C, has been measured using methods previously described (Brown & Halliwell, 1972). As shown in Table 1, other bases tended to give pH_i values similar to those of nicotine, in partial confirmation of predictions.

It may also be noted that, because of cell acidity, basic drugs may accumulate in these cells to a much higher concentration than in the surrounding medium. This may have implications for their pharmacology and toxicity.

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References

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WADDELL, W.J. & BUTLER, J.V. (1959). Calculation of intracellular pH from the distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO). Application to skeletal muscle of the dog. *J. clin. Invest.*, **38**, 720-729.

Table 1 Characteristics of the uptake of some weak acids and bases into isolated rat sympathetic ganglia

	pKa at 25° C	*Equilibrium time (min)*	*Concentration range (μM)*	*Ci/Co at equilibrium*	*Calculated pH_i*
A. Weak bases					
[^{3}H]-Nicotine	8.01	30	0.096-60	6.172 ± 0.116(20)	6.494 ± 0.009
[^{3}H]-Atropine	9.71	90	0.34-100	7.097 ± 0.155(21)	6.539 ± 0.013
[^{14}C]-Morphine	7.94	90	0.35-1000	6.084 ± 0.136(36)	6.507 ± 0.011
[^{14}C]-Procaine*†	8.91	60	2000	5.228 ± 0.287(4)	6.636 ± 0.013
[^{14}C]-Trimethylamine*	9.81	60	1000-2900	3.180 ± 0.131(10)	6.935 ± 0.023
B. Weak acids					
[^{14}C]-DMO	6.33	30	110	0.873 ± 0.007(46)	7.311 ± 0.003
[^{14}C]-Phenobarbitone	7.45	30	8.0-3300	2.664 ± 0.051(18)	Ca 8.0

Results given as mean ± s.e. mean. Number of ganglia used indicated in brackets.
* Values given for concentrations which give apparent saturation of binding components.
† Physostigmine (10^{-5} g/ml) was included in the bathing media to prevent metabolism of procaine.
Ci/Co refers to intracellular to extracellular concentration ratio.