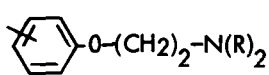# INHIBITION OF PROTOZOAN MOTILITY BY LOCAL ANAESTHETICS

D-A.M.A. Al-Saadi, A.J. Baillie, W. Farrell, W.E. Sneader, N.G. Waton, School of Pharmaceutical Sciences, University of Strathclyde, Glasgow, G1 1XW.

The inhibitory activity of some inhalational anaesthetics on the swimming speed of the ciliate Tetrahymena pyriformis correlates with their potency in animals (Nunn et al 1974) and we describe here an inverse correlation between the minimum inhibitory concentration (MIC) of drug required to immobilise T. pyriformis cells and the duration of local anaesthesia for several novel local anaesthetics of the general structure R'—⬡—O–$(CH_2)_2$–$N(R)_2$ used throughout as hydrochlorides.

3 day old T. pyriformis cells, GL strain maintained in unshaken axenic culture at room temperature in Neopeptone broth (Difco) were washed twice in 6.7 mM pH 7.2 phosphate buffer by centrifuging ($\fallingdotseq$ 80g for 4 min) and left overnight in fresh buffer. In the flat bottomed wells of a micro-titre plate, 0.2ml volumes of buffered cell suspension (5×10^4 cells ml^{-1}) were mixed with 0.2ml buffered anaesthetic solutions in a range of concentrations such that the lowest concentration (MIC) which completely inhibited motility after 60 min could be determined by examining the wells microscopically (160 x magnification). The duration of action of the anaesthetics (WT), tested as 0.5% w/v solutions in the guinea pig intradermal wheal test (Bulbring & Wajda 1945) and their partition coefficients (log P) in octanol/pH 7.4 phosphate buffer (Fujita et al 1964) were also determined.

Table 1.   Anaesthetic MIC (% w/v), WT (h), and log P

R'	R	MIC	WT	log P	R'	R	MIC	WT	log P
2,6-(t-Bu)$_2$-4-Me	Me	< 0.1	> 24	2.07	4-(s-Bu)	Me	< 0.125	$\leqslant$ 24	1.05
2,6-(s-Bu)$_2$	Me	< 0.1	> 24	1.45	2,4,6-(Me)$_3$	Me	0.1-0.2	$\leqslant$ 4	0.18
4-(t-AM)	Me	< 0.1	$\leqslant$ 6	1.32	3,5-(Me)$_2$	Me	0.2-0.4	$\leqslant$ 4	0.79
2,6-(t-Bu)$_2$	Me	< 0.1	> 24	1.00	2,6-(Me)$_2$	Et	0.2-0.4	3	0.75
2,4,6-(Cl)$_3$	Me	< 0.1	3	1.08	3-OEt	Me	0.4-0.6	3	0.86
4-(n-OBu)	Me	< 0.125	> 24	1.46	3-OMe	Et	0.4-0.6	2	0.84
4-(t-Bu)	Me	< 0.125	$\leqslant$ 24	1.22	Lignocaine		1.5-2.0	1	1.13

The Spearman rank correlations (r_s) for the data, excluding that for lignocaine are: between MIC and WT, $r_s = 0.67$, $P < 0.01$; MIC and log P, $r_s = 0.71$, $P < 0.0005$; WT and log P, $r_s = 0.70$, $P < 0.01$. The dependence of both MIC and WT on log P indicates that in both biological systems the anaesthetics interact with hydrophobic structures, presumably the cell membrane in T. pyriformis resulting in fluidisation and loss of ciliary function. Further evidence for the involvement of the cell membrane was the cell clumping and swelling seen at high concentrations of some agents and it would be interesting to attempt to relate these phenomena to their irritancy or toxicity. This simple inexpensive test can thus discriminate between local anaesthetics of different duration of action.

Bulbring, E. and Wajda, I (1945). J. Pharmacol. 85, 78-84
Fujita, T. et al (1966). J. Am. Chem. Soc. 86, 5175-5180
Nunn, J.F. et al (1974). J. Cell Sci. 15, 537-554