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The anticholinesterase activity of pancuronium bromide (Pavulon®)

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PANCURONIUM (2β,16β-dipiperidino-5α androstan-3α17β-diol diacetate dimetbromide), a bis-quaternary derivative of androstan, also having two acetyl radicals, is a nondepolarizing, competitive, neuromuscular blocking agent (Fig. 1). Its anticholinesterase activity has been reported recently in a few clinical and experimental studies.[5–7] These observations are not surprising because many structure–activity relationship studies with bis-onium neuromuscular blocking agents[8,9] have shown their

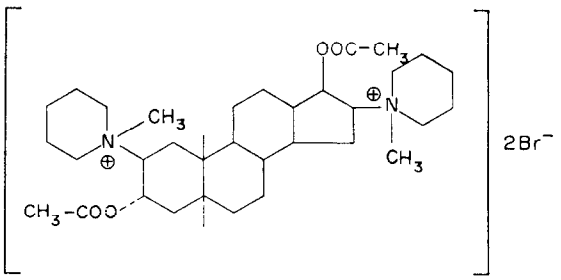

FIG. 1. Structure of pancuronium bromide.

relatively high anticholinesterase activity, which is not related to the type of neuromuscular block. Pancuronium is particularly interesting from this point of view, having an interonium distance like *d*-tubocurarine and containing a bulky steroid radical between these two onium groups.

Thus, we thought it worthwhile to study the effect of pancuronium on the different cholinesterases as well as on the kinetics of their inhibition. The spectrophotometric method of Ellman *et al.*[10] was used to measure cholinesterase activity. Enzyme and inhibitor were incubated at 25°, in 100 mM phosphate buffer, pH 7·4, containing 0·2 mM DTNB (5,5′-dithiobis-2 nitrobenzoic acid) in a final volume of 1 ml. The substrates (acetylthiocholine or butyrylthiocholine) were added in small volumes to give a final concentration of 0·03–0·50 mM and the absorption increase at 412 nm was recorded with a Zeiss VSU-1 model spectrophotometer.

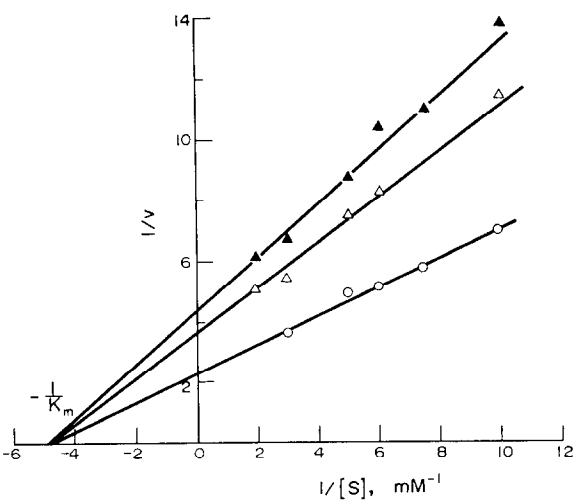

FIG. 2a

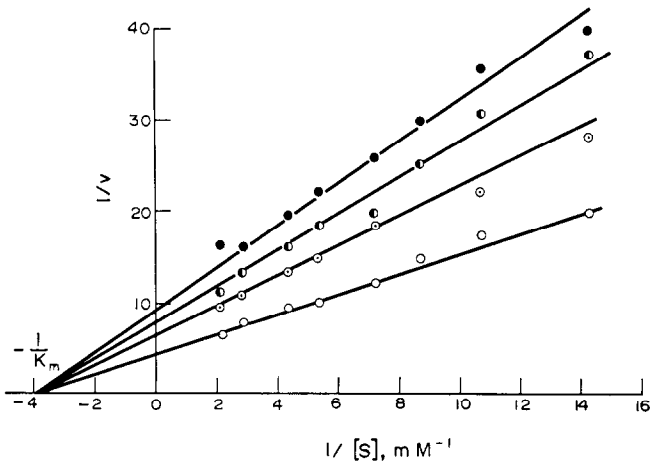

FIG. 2b

FIG. 2. Inhibition of erythrocyte acetylcholinesterase (a) and serum cholinesterase (b) by pancuronium. 0·01 mg enzyme preparation, dissolved in 100 mM phosphate buffer, pH 7·4, was used in each experiment. The reaction velocities are expressed as ΔE/min. At 412 nm, 1 ml final volume and 1 cm light path length, an extinction increase of 0·1 corresponds to 7·35 nmoles hydrolysed substrate. (○) without inhibitor; (⊙) 1·67 μM pancuronium; (◐) 3 μM pancuronium; (●) 5 μM pancuronium; (△) 30 μM pancuronium; (▲) 50 μM pancuronium.

Preliminary experiments with purified bovine erythrocyte acetylcholinesterase (Sigma Chem. Corp. St. Louis) and horse serum cholinesterase (Schuchardt, GmbH, München), showed that pancuronium inhibits both enzymes by an equilibrium reaction which is practically time-independent. Figures 2a, b represent the slope of $1/v$ vs $1/[S]$ for erythrocyte acetylcholinesterase and serum cholinesterase at different concentrations of pancuronium. Pancuronium does not modify the K_m values of these enzymes, but it decreases V_{max}. In experiments where the substrates were added to the reaction medium before adding the inhibitor there was no protection against the inhibition. Inhibitor constants (K_i) determined from the values of the appropriate intercepts of Lineweaver–Burk plots, both in the presence and in the absence of pancuronium[11] were of 67·0 $\pm$ 4·8 μM for erythrocyte acetylcholinesterase and 4·1 $\pm$ 0·1 μM for serum cholinesterase.

We conclude that pancuronium is a pure noncompetitive inhibitor of erythrocyte and serum cholinesterases, unlike *d*-tubocurarine or other bis-onium salts, which were shown to be competitive blockers of both the acetylcholinesterase and the cholinergic receptors of the electric eel.[12] On the other hand, K_i values show that pancuronium is a weaker inhibitor of erythrocyte acetylcholinesterase and a relatively strong inhibitor of serum cholinesterase. Therefore, the neuromuscular blocking doses in man (0·04–0·10 mg/kg) probably do not produce the required concentration for erythrocyte acetylcholinesterase inhibition, but the inhibition of serum cholinesterase cannot be excluded.

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Behaviour of an aziridine alkylating agent in acid solution

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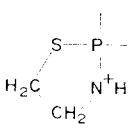
Tris(1-aziridinyl)phosphine sulphide (thiotepa) I, a biological alkylating agent used clinically in the treatment of breast cancer, is inactive when administered orally. It is unstable at pH 4·2 at 37°[1,2] and the rapid inactivation of a related compound, 2,4,6-triaziridinyltriazine, in acid solution is also recorded.[3] At low pH thiotepa is said to undergo an intramolecular alkylation giving a ring structure of the type.[4]



Since we had already observed the instability of thiotepa in acid solution it was decided to try to obtain additional evidence for the intramolecular alkylated product.

Isotopically labelled compounds, ^{35}S thiotepa, ^{32}P thiotepa and ^{36}Cl sodium chloride were obtained from the Radiochemical Centre, Amersham. Unlabelled thiotepa was supplied by Lederle Ltd. 1 ml M HCl was added to 20 ml of solutions of thiotepa in 0·2 M NaCl.[4] Changes in pH of these solutions were followed using a glass electrode and samples were removed at fixed time intervals for chromatographic examination. Thin-layer chromatography investigations were performed on silica gel plates using *n*-butanol saturated with ammonia. Spots were visualized using 5% 4-(4-nitrobenzyl)pyridine (NBP) in acetone and Ninhydrin. Radioactivity was detected using a geiger counter mounted on a travelling microscope base and autoradiography. The presence of hydrogen sulphide was confirmed using the British Pharmacopoeia arsenic limit test apparatus[5] but omitting moistening of the cotton wool with lead acetate solution and replacing the mercuric chloride paper with lead acetate paper.

Aqueous thiotepa solutions produce a single, blue spot (R_f 0·67), visualized using NBP. Solutions of thiotepa in saline produced two spots (R_f 0·67 and R_f 0·77), both of which were NBP positive and therefore contained an alkylating function. When ^{32}P thiotepa (1 mg/ml) in saline was used, these two spots were radioactive. However, when non-radioactive thiotepa solutions were prepared in ^{36}Cl saline only the spot (R_f 0·77) was radioactive, suggesting a chloro-derivative of thiotepa. This has been shown to be the monochloro-derivative III.[6]

Acidification of thiotepa solutions in saline was followed by a concentration dependent pH rise. For example, solutions containing 5 mg/ml thiotepa showed a pH rise from 1·5 to 6·0. In contrast,