IN VITRO INTERACTION OF PROPANIDID AND SUXAMETHONIUM WITH POOLED HUMAN PLASMA CHOLINESTERASE

A kinetic study

P. E. TAUSSIG, H. E. STOJAK AND N. R. BENNETT

SUMMARY

Human plasma cholinesterase (E.C. 3.1.1.8) was shown to be inhibited by physiological concentrations of propanidid and suxamethonium using a colourimetric assay at 25 °C and pH 7.2 unit with butyrylthiocholine as substrate. Propanidid inhibited the enzyme in a non-competitive manner ($I_50 = 2.0 \text{ mmol litre}^{-1}$; apparent $K_m = 6.6 \times 10^{-4} \text{ mol litre}^{-1}$) as did suxamethonium ($I_50 = 4.4 \text{ mmol litre}^{-1}$; apparent $K_m = 1.6 \times 10^{-4} \text{ mol litre}^{-1}$). Combined inhibition produced $K_m = 3.0 \times 10^{-3} \text{ mol litre}^{-1}$. The binding of these drugs to specific anionic sites in the vicinity of the active centre is thought to result in stereochemical changes in the enzyme. This mechanism and its relevance to the augmentation of the neuromuscular blockade produced by suxamethonium in the presence of propanidid is discussed.

There is evidence (Doenicke et al., 1968; Ellis, 1968; Kraunak, Pleurvry and Rees, 1977) which points to the involvement of a cholinesterase enzyme in the metabolism of the short-acting anaesthetic agent propanidid (propyl-4-NN-diethylcarbamoylmethoxy-3-methoxyphenylacetate). In human plasma the enzyme most likely to be involved in the hydrolysis of both propanidid and suxamethionium is pseudocholinesterase (E.C. 3.1.1.8) (Doenicke, Schmidinger and Krumey, 1968).

The loss of the anaesthetic potency of propanidid has been ascribed to cleaving of the n-propyl ester linkage (Doenicke et al., 1968), thereby producing an acid devoid of biological effect. In vivo studies have shown that propanidid prolonged the duration of action of suxamethonium (Doenicke et al., 1968) which itself acted as a substrate for plasma cholinesterase (Doenicke, Schmidinger and Krumey, 1968). In a recent study, Doenicke and others (1968) showed that the period of apnoea produced by suxamethonium was prolonged by more than 3 min in the presence of propanidid. This potentiation by suxamethonium has been the subject of a number of in vitro studies by Ellis (1968), Kraunak, Pleuvry and Rees (1977) and others who have sought to elucidate the nature of the interaction between propanidid, suxamethonium and neuromuscular blockade.

Ellis (1968), using a rat diaphragm preparation, showed elegantly that the duration of neuromuscular blockade produced by suxamethonium was potentiated by propanidid in a dose-related manner. It was shown also that this potentiation occurred in the presence of mipaflox which completely inactivates cholinesterase at the neuromuscular junction. Thus it was postulated that propanidid could potentiate the effects of suxamethonium by a peripheral effect on the muscle cell membrane and that propanidid had little demonstrable anticholinesterase activity. In addition, propanidid could potentiate the neuromuscular blockade produced by tubocurarine.

Subsequent work has demonstrated that serum cholinesterase may be inhibited by pancuronium (Stovner, Ofstedal and Holmboe, 1975) and ketamine (Schuh, 1975a). Stovner, Ofstedal and Holmboe (1975) provided evidence that red cell acetycholinesterase was inhibited by pancuronium. The biological effect of pancuronium was potentiated by a number of i.v. agents in nerve-muscle preparations. The influence of ketamine on human plasma cholinesterase was studied by Schuh (1975a), who was able to show that the anaesthetic interacted in a non-competitive manner with the enzyme in vitro.

Despite the volume of data pertaining to the clinical evaluation of the potentiating effects of i.v. anaesthetic agents on the non-depolarizing myoneural blocking drugs, few attempts have been made to explore these interactions at the molecular level. Although the interaction between ketamine and pancuronium with human cholinesterases has been examined in vitro there has been no study of a combination of suxamethonium and propanidid.

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Plasma cholinesterase is important clinically when patients are exposed to myoneural blocking drugs and i.v. agents (Schuh, 1975b). In the present study the interactions between serum cholinesterase (E.C. 3.1.1.8), suxamethonium and propanidid were studied in vitro.

METHODS

Assay
The assay method was dependent on the ability of the enzyme butyrylcholinesterase to cleave butyrylthiocholine. Thiocholine, liberated in this reaction, reacts with 5,5'-dithiobis-2-nitrobenzoic acid which is present in a phosphate buffer solution to produce 5-mercapto-2-nitrobenzoate, which possesses a yellow colour. The change in optical density at 405 nm per unit time is a measure of butyrylcholinesterase activity, and was detected using a Beckmann DB GT spectrophotometer.

Butyrylthiocholine iodine and 5,5'-dithiobis-2-nitrobenzoic acid were obtained from British Drug Houses. Butyrylcholinesterase was obtained by pooling plasma from six healthy volunteers and storing it at −20 °C in 2-ml aliquots. Using a pipette, 3.0 ml of phosphate buffer (pH 7.20 unit) was introduced to the spectrophotometer cuvette followed by 0.1 ml of butyrylthiocholine solution. To this mixture was added 0.02 ml of plasma, the change in optical density was recorded using a Beckmann spectrophotometer and the results were displayed on a potentiometric chart recorder.

Suxamethonium and propanidid
The effects of separately varying concentrations of suxamethonium and propanidid on the reaction between butyrylthiocholine and butyrylcholinesterase were assessed. In addition, the effects of varying combinations of suxamethonium and propanidid in the reaction between substrate and enzyme were studied.

Dilutions of commercially available propanidid solubilized in 20% Cremophor EL (Bayer Pharmaceuticals) and suxamethonium (succinyldicholine, Burroughs–Wellcome Ltd) were made using distilled water.

Mathematical formulations
Fundamental Michaelis–Menten kinetics were applied to this enzyme:

\[ \frac{V}{V_{\text{max}}} = \frac{S}{K_m + S} \]

where

\[ V = \text{velocity of reaction at any substrate concentration } S, \]
\[ V_{\text{max}} = \text{maximal attainable velocity when enzyme is saturated with substrate,} \]
\[ K_m = \text{Michaelis–Menten constant.} \]

Rates of reaction were measured in terms of the change in optical density per second and lines were plotted from the statistical calculations employing the method of least squares.

RESULTS
Human plasma cholinesterase was inhibited by propanidid in a concentration-dependent manner. The inhibitor \((V_0/V_1 \text{ v. } I)\) plot (fig. 1) for propanidid shows that the enzyme is 50% inhibited at a propanidid concentration of 2.0 mmol litre\(^{-1}\).

![Fig. 1. Inhibitor \((V_0/V_1 \text{ v. } I)\) plot for human plasma cholinesterase with propanidid, where \(V_0 = \text{rate of enzyme hydrolysis in the absence of any inhibitor and } V_1 \text{ is the rate of hydrolysis in the presence of propanidid at each concentration of } I. \text{ Propanidid concentration for half maximal inactivation at 2.0 mmol litre}^{-1}.\]

The same plot for suxamethonium inhibition (fig. 2) yielded a value of suxamethonium 4.4 mmol litre\(^{-1}\) for half-maximal inactivation. The Lineweaver–Burk \((1/V \text{ v. } 1/S)\) plot for plasma cholinesterase in the absence of any inhibitor is shown in figure 3. The enzyme had \(K_m\) of \(1.2 \times 10^{-4}\) mol litre\(^{-1}\).

The addition of 100 μl of undiluted propanidid to the buffer (corresponding to a molar concentration of anaesthetic in the cuvette of 4.04 × 10^{-3} mol litre\(^{-1}\)) inhibited the enzyme activity by more than 66%. Extrapolation of the line, produced by plotting the reciprocals of rate and substrate concentration...
INTERACTIONS WITH PLASMA CHOLINESTERASE

0.62 1.24 1.86 2.48 ai 3.72 4.96
SUXAMETHONIUM CONCN (mmol litre$^{-1}$)

FIG. 2. Inhibitor ($V_0/V_1$ v. $I$) plot for human plasma cholinesterase with suxamethonium. (See legend to figure 1 for explanation of symbols.) Suxamethonium concentration at half-maximal inactivation at 4.4 mmol litre$^{-1}$.

(fig. 3, a) for enzyme inhibited by propanidid, revealed that a 10-fold decrease in $K_m$ had been produced by the addition of the anaesthetic agent. Exposure of the enzyme to 100-μlitre aliquots of propanidid diluted 50- and 200-fold with distilled water produced the same pronounced effect on $K_m$ (fig. 3, b and c, and table I). In addition, the value of $V_{max}$ at each concentration of propanidid which is shown by the intercept which each plot makes with the $y$-axis, was altered in all three instances. These data suggested that the interaction of propanidid with the enzyme was essentially of the non-competitive type.

Human plasma cholinesterase is shown to be inhibited by suxamethonium (figs 2 and 4, table I). $K_m$ of the enzyme in the presence of suxamethonium

<table>
<thead>
<tr>
<th>$K_m$ (mol litre$^{-1}$)</th>
<th>$V_{max}/K_m$</th>
<th>Inhibitor (mol litre$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6 x 10^{-4}</td>
<td>3.2 x 10^3</td>
<td>Propanidid 4.04 x 10^{-3}</td>
</tr>
<tr>
<td>1.6 x 10^{-4}</td>
<td>12.5 x 10^3</td>
<td>Suxamethonium 12.4 x 10^{-3}</td>
</tr>
<tr>
<td>3.3 x 10^{-4}</td>
<td>6.0 x 10^3</td>
<td>Propanidid and suxamethonium 2.02 x 10^{-3}</td>
</tr>
<tr>
<td>8.3 x 10^{-5}</td>
<td>2.4 x 10^4</td>
<td>Control—no inhibitor</td>
</tr>
</tbody>
</table>

Fig. 3. Lineweaver-Burk ($1/V$ v. $1/S$) plot for human plasma cholinesterase in the absence of any inhibitor $\Delta$. Values of $K_m$ and $V_{max}$ were obtained from the intercepts the line made with the $x$- and $y$-axes. (See text for explanation of symbols.) a, b and c: Lineweaver-Burk ($1/V$ v. $1/S$) plots for human plasma cholinesterase in the presence of propanidid. $\bigcirc =$ Propanidid 4.04 x 10^{-3} mol litre$^{-1}$; $\bullet =$ propanidid 8.1 x 10^{-4} mol litre$^{-1}$; $\bigodot =$ propanidid 2.2 x 10^{-4} mol litre$^{-1}$. Lines were plotted using the least squares method of regression analysis.
The Lineweaver-Burk \((1/V \text{ v. } 1/S)\) plot for human plasma cholinesterase in the presence of suxamethonium. \(\circ = \text{suxamethonium } 12.4 \text{ mmol litre}^{-1}; \odot = \text{suxamethonium } 0.49 \text{ mmol litre}^{-1}; \bullet = \text{suxamethonium } 0.248 \text{ mmol litre}^{-1}.\) Lines were plotted using the least squares method of linear regression analysis.

An \textit{in vitro} concentration of propanidid of \(4.04 \times 10^{-3}\) mol litre\(^{-1}\) produced a 66\% inhibition of the enzyme under the conditions of the assay using the spectrophotometer. It was possible to detect inhibition of the enzyme at \textit{in vitro} concentrations of propanidid as little as \(2 \times 10^{-5}\) mol litre\(^{-1}\). These concentrations compare with the calculated \textit{in vivo} values of \(3.14 \times 10^{-4}\) mol litre\(^{-1}\) assuming 0.5 g of propanidid to be distributed uniformly in 4 litre of plasma, or \(8 \times 10^{-5}\) mol litre\(^{-1}\) which is equivalent to the concentration of the same dose of propanidid in 15 litre of extracellular fluid.

Inhibition of butyrylcholinesterase was observed at the \textit{in vitro} concentration of suxamethonium of \(10^{-5}\) mol litre\(^{-1}\), which compares with the calculated \textit{in vivo} values of \(8.7 \times 10^{-5}\) mol litre\(^{-1}\) and \(2.31 \times 10^{-5}\) mol litre\(^{-1}\) for blood and extracellular fluid respectively following a 100-mg bolus of suxamethonium.

However, these calculations are only estimates, and do not take into account factors such as protein-binding and ionization of the relevant drugs. It should be remembered also that the drug is exposed only to a finite quantity of enzyme in the spectrophotometer cuvette. \textit{In vivo} the serum concentration of enzyme may exceed that of the cuvette by 150-fold. Attempts to demonstrate enzyme inhibition \textit{in vivo} following the i.v. administration of 0.5 g of propanidid were unsuccessful (Alderson and Taussig, unpublished).
results, 1977). In contrast, Doenicke and others (1968) found that plasma cholinesterase activity was depressed by 20% following a similar exposure of the enzyme to propanidid in a normal volunteer. Clearly, individual differences exist in the ability to metabolize propanidid, but the high rate of clearance of this drug from the plasma by pseudocholinesterase and liver esterases makes the detection of in vivo inhibition a tenuous possibility.

We can only speculate as to the molecular mechanism underlying the potentiation of the action of suxamethonium by propanidid. The active centre of the cholinesterases contains two sites: an esteratic site and an anionic site (Bergmann, 1955). There are additional anionic sites around the periphery of the active site. Interactions of drugs bearing positively-charged moieties with these peripheral anionic sites may cause conformational changes in the active centre (Koshland, 1960, 1963). Conversely, conformational changes at the active centre may induce stereochemical changes in these anionic sites, making them less available for interactions with drugs bearing positively-charged groups, such as suxamethonium.

Therefore, prolongation of the action of suxamethonium by propanidid may be a result of the binding of propanidid to an anionic site, inducing a stereochemical distortion, trapping of the substrate in the active centre and either hindering the hydrolysis reaction or, possibly, the release of products from the active site. This would account for the predominantly non-competitive nature of the kinetics of the propanidid–cholinesterase interaction. The binding of suxamethonium, in the absence of propanidid, to a specific anionic site on the enzyme may cause a similar type of inhibition of function except that the degree of conformational change induced thereby would be less severe in view of the fact that suxamethonium is a less potent inhibitor of the enzyme than is propanidid. In the presence of both propanidid and suxamethonium the complex combination of stereochemical changes that results is sufficient to cause impairment of the binding of suxamethonium to the enzyme. In the clinical situation this would result in a greater proportion of the injected suxamethonium being made available at the neuromuscular junction with prolongation of suxamethonium action.

Mechanistic considerations apart, it may be concluded that plasma cholinesterase may be only partially responsible for the breakdown of suxamethonium and propanidid in man and that the interactions of these drugs with liver esterases and the cholinesterases of the neuromuscular junction may be the dominant mechanism.

REFERENCES


INTERACTION IN VITRO DU PROPANIDID ET DU SUXAMETHONIUM AVEC CHOLINESTERASE DU PLASMA HUMAIN MIS EN COMMUN

Etude cinétique

RESUME

Il a été montré que la cholinesterase du plasma humain (E.C. 3.1.1.8) pouvait être inhibée par les concentrations physiologiques de propanidid et de suxaméthonium, en faisant une analyse colorimétrique à 25 °C et à un pH de 7,2, en utilisant comme substratum de la butyrylthiocholine. Le propanidid a inhibé l'enzyme d'une manière non compétitive ($K_m$ apparent = 6,6 x 10^-4 mmol litre^-1), tout comme le suxaméthonium ($I_50$ = 4,4 mmol litre^-1; $K_m$ apparent = 1,6 x 10^-4 mmol litre^-1). L'inhibition combinée a donné un $K_m$ de 3 x 10^-3 mmol litre^-1. La liaison de ces médicaments sur certains sites anioniques spécifiques à proximité du centre actif résulte, existe-t-on, des changements stéréochimiques de l'enzyme. On décrit dans cet article, ce mécanisme de même que son influence sur l'augmentation de blocage musculaire produit par le suxaméthonium en présence de propanidid.
IN VITRO WECHSELWIRKUNG VON PROPANIDID UND SUXAMETHONIUM MIT ANGESAMMELTER, MENSCHLICHER PLASMACHOLINESTERASE

Kinetische Untersuchung

ZUSAMMENFASSUNG

Durch eine kolorimetrische Analyse wurde gezeigt, dass menschliche Plasmacholinesterase (E.C. 3.1.1.8) durch physiologische Konzentrationen von Propanidid und Suxamethonium gehemmt wurde. Die Analyse wurde bei 25 °C und einer pH von 7,2 Einheiten Butyrylthiocholin als Substrat gemacht. Propanidid hemmte das Enzym in einer nichtkonkurrierenden Weise (Hemmer 60 = 2,0 mmol liter⁻¹; sichtbare $K_m = 6,6 \times 10^{-4}$ mol liter⁻¹), und so auch Suxamethonium (Hemmer 60 = 4,4 mmol liter⁻¹; sichtbare $K_m = 1,6 \times 10^{-4}$ mol liter⁻¹). Eine vereinte Hemmung produzierte eine $K_m$ von $3,0 \times 10^{-3}$ mol liter⁻¹. Das Binden dieser Drogen an spezielle anionische Regionen in der Nähe des aktiven Zentrums, so denkt man, ergibt stereochemische Änderungen im Enzym. Dieser Mechanismus und seine Bedeutung auf die Verstärkung der neuromuskulären Blockade, die durch Suxamethonium im Beisein von Propanidid produziert wurde, wurde diskutiert.

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INTERACCIÓN IN VITRO DE PROPANIDIDA Y SUXAMETONIO CON COLINÉSTERASA REUNIDA DE PLASMA HUMANA

Un estudio cinético

SUMARIO

Se demostró que la colinesterasa (E.C. 3.1.1.8) en la plasma humana es inhibida por concentraciones fisiológicas de propanidida y suxametonio, empleando una prueba colorimétrica a 25 ºC y una unidad de pH 7,2 con butirilothiocolina en calidad de substrato. La propanidida inhibió a la encima de forma no competitiva ($I_{60} = 2,0$ mmol litro⁻¹; $K_m$ aparente = $6,6 \times 10^{-4}$ mol litro⁻¹), así como lo hizo el suxametonio ($I_{60} = 4,4$ mmol litro⁻¹; $K_m$ aparente = $1,6 \times 10^{-4}$ mol litro⁻¹). La inhibición combinada produjo un $K_m$ de $3,0 \times 10^{-3}$ mol litro⁻¹. Se piensa que la unión de estas drogas a determinados sitios aniónicos próximos al centro activo, da por resultado cambios estereoquímicos en la encima. Se discute este mecanismo y su relevancia al acrécentamiento del bloqueo neuromuscular producido por suxametonio en presencia de propanidida.