Effects of Trimethaphan and Sodium Nitroprusside on Hydrolysis of Succinylcholine in Vitro

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The use of hypotensive agents in combination with succinylcholine may be necessary. Since trimethaphan has been reported to prolong the action of succinylcholine, the authors studied the abilities of trimethaphan and sodium nitroprusside to inhibit hydrolysis of succinylcholine by pseudocholinesterase in vitro. Trimethaphan was found to be a potent noncompetitive inhibitor of pseudocholinesterase (K_i = 0.24 μM). It could be calculated that a typical dose of trimethaphan would approximately double the duration of paralysis produced by the usual dose of succinylcholine. Nitroprusside had no inhibitory effect in vitro. It is concluded that nitroprusside is preferable when a hypotensive agent must be used in conjunction with succinylcholine. (Key words: Neuromuscular relaxants, succinylcholine; Enzymes, pseudocholinesterase; Anesthetic techniques, hypotension, induced, trimethaphan; Anesthetic techniques, hypotension, induced, nitroprusside.)

VARIous INVESTIGATORS have demonstrated an action of trimethaphan on neuromuscular blockade,1-4 and have warned against simultaneous use of trimethaphan and succinylcholine. Tewalk5 reported prolonged apnea in patients treated with these two drugs, and suggested that trimethaphan was metabolized by pseudocholinesterase, thereby competitively inhibiting the hydrolysis of succinylcholine and prolonging its action. Gertner et al.,6 however, found that trimethaphan is probably not metabolized by pseudocholinesterase. As the potential exists for hypotensive agents to be used in conjunction with succinylcholine, we decided to study in vitro the possibility of synergistic effects due to inhibition of pseudocholinesterase when succinylcholine and either trimethaphan or sodium nitroprusside are administered simultaneously.

Methods

Serum for all experiments was obtained by centrifugation of coagulated blood from various random donors whose pseudocholinesterase levels were normal. Enzymatic activity was determined using butyrylthiocholine (Eastman Kodak Co.) as substrate and 5,5'-dithiobis(2-nitrobenzoic) acid as the color reagent in a buffer containing 0.01 M 3-(N-morpho-

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line)-propanesulfonic acid (pH 7.60).7 Absorbance change at 412 nm was continuously monitored using a spectrophotometer and recorder (Gilford Instrument Laboratories, Oberlin, Ohio). Trimethaphan (Hoffman-La Roche), succinylcholine (Burroughs Wellcome), and sodium nitroprusside (Hoffmann-La Roche) were added to the reaction system from concentrated stock solutions to achieve the desired final concentrations. In one series of experiments, the ability of trimethaphan to inhibit the hydrolysis of procaine was determined by using the decrease in absorbance at 313 nm as a measure of the rate of substrate hydrolysis.8

Data from these inhibition experiments were plotted in two ways. First, reciprocal of the reaction velocity versus reciprocal of the substrate concentration was used to determine the type of inhibition and, second, reciprocal of the reaction velocity versus inhibitor concentration was used to determine K_i, the dissociation constant for the inhibitor. The Michaelis constant, K_M, for substrate was determined from the usual double-reciprocal plot. In all cases, lines were drawn through the data points using a linear least-squares approximation. The concentration of inhibitor giving 50 per cent inhibition (I_50) is given by the expression, I_50 = K_i (K_M + S)/K_M. It was not possible for us to measure K_M for succinylcholine directly, but a K_i could be calculated from its effect as an inhibitor of butyrylthiocholine hydrolysis. Since K_i and K_M are analogous kinetically determined measures of the affinities of the enzyme for inhibitor and substrate, respectively, this K_i was then used in the place of K_M to estimate the effect of trimethaphan on succinylcholine hydrolysis.

Results

The reciprocal of the rate of butyrylthiocholine hydrolysis was determined at various concentrations of trimethaphan. The data points obtained at constant concentrations of substrate could be fitted by straight lines that intersect at a point lying above the X axis and to the left of the Y axis (Fig. 1). Using the method of joint saturations,9 in which the enzyme is simultaneously saturated with both inhibitor and substrate, it appeared that the catalytic efficiency of the enzyme-substrate-inhibitor complex was about 12 per cent that of the enzyme-substrate complex in the absence of inhibitor.

This pattern of inhibition is mixed noncompetitive in type,9 and the K_i obtained from the intersec-
tion indicated in figure 1 is 0.24 μM. When procaine was used as substrate the $K_i$ for trimethaphan inhibition was found to be 0.13 μM. Because of the small change in extinction coefficient and the relatively slow reaction rate when procaine was used as substrate, the $K_i$ obtained using butyrylthiocholine was more precise, and was used in subsequent calculations. Similarly, the $K_i$ for succinylcholine inhibition of butyrylthiocholine hydrolysis was found to be 15.1 μM in our system. The $K_M$ for butyrylthiocholine itself was found to be 0.10 mM, in fairly good agreement with values of 0.06 mM$^{10}$ and 0.07 mM$^{11}$ obtained from the usual genetic variant of pseudocholinesterase in other buffer systems.

In contrast to the potent inhibitory effect of trimethaphan, concentrations of nitroprusside as high as $3.3 \times 10^{-4}$ M produced no inhibition of pseudocholinesterase even when assayed at the relatively low butyrylthiocholine concentration of $2 \times 10^{-4}$ M. These data suggest that nitroprusside does not significantly inhibit the hydrolysis of succinylcholine in vivo.

**Discussion**

We find that trimethaphan is a potent inhibitor of pseudocholinesterase in vitro. Is the inhibition sufficient to prolong the action of succinylcholine when the two agents are administered simultaneously? Succinylcholine, 80 mg, distributed in 10 liters of extracellular water achieves a concentration of approximately 0.2 μM. Taking the kinetic constants described above, the concentration of trimethaphan needed to produce 50 per cent inhibition of the rate of succinylcholine hydrolysis is

$$0.24 \mu M \left[\frac{(15.1 \mu M + 0.2 \mu M)}{15.1 \mu M}\right] = 0.24 \mu M$$

Assuming trimethaphan and succinylcholine to be distributed in the same volume, this concentration would be achieved by a dose of 1.4 mg. In our experience, this dose is within the range typically needed to induce hypotension.

Thus, a typical dose of trimethaphan would be expected to double the duration of paralysis produced by a usual dose of succinylcholine. These in vitro findings are consistent with the clinical observations of Tewfik$^{2}$ indicating prolongation of neuromuscular blockade by that combination of drugs. However, the mechanism of pseudocholinesterase inhibition is noncompetitive, implying that binding of the inhibitor does not completely preclude binding of substrate.$^9$ No such action is predicted when succinylcholine and nitroprusside are administered in combination. In fact, when we administered this combination to a hypertensive patient, we failed to find any prolongation of paralysis.

The mechanism by which the action of trimethaphan is terminated is not yet understood. Since it is not an ester, and since an average of 31 per cent of
administered trimethaphan is recovered, unchanged and still biologically active, in the urine, it is probably not metabolized by pseudocholinesterase. Clearly, the disposition of this drug requires further study.

Induced hypotension may be necessary from time to time for surgical reasons. It may also be needed for anesthetic purposes, for example, to prevent a noxious increase in blood pressure during laryngoscopy and intubation in a patient paralyzed with succinylcholine. This reaction has long been known, and occurs frequently. We became interested in using hypotensive agents with induction of endotracheally administered anesthesia following adverse hypertensive responses in two of our patients, one of whom had acute pulmonary edema, and the other a ruptured cerebral aneurysm, after anesthetic induction. Both of these patients were chronically hypertensive.

Nitroprusside and trimethaphan were evaluated because they are rapid-acting, yet short-acting, hypotensive agents. Since they are administered intravenously, their actions can be limited and discontinued by controlling the speed of infusion. On the basis of our results in vitro, we feel that nitroprusside is preferable when a hypotensive agent must be administered in combination with succinylcholine.

References


