Laboratory Investigations

Pancuronium, vecuronium, and d-tubocurarine inhibit and succinylcholine stimulates choline acetyltransferase activity

J. Reddy Kambam MD,* V.E. Janson,†
Paula Day,† B.V.R. Sastry PhD†

The effects of the nondepolarizing muscle relaxants (NDMR), pancuronium, vecuronium, and d-tubocurarine and a depolarizing muscle relaxant, succinylcholine, were studied on choline acetyltransferase (ChAT) activity. A radiochemical assay was used in the determination of ChAT activity using purified placental enzyme. Pancuronium at concentrations of 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M inhibited ChAT activity by 3, 10, 15, 40 and 85 per cent, respectively; vecuronium at concentrations of 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M inhibited ChAT activity by 5, 10, 26 and 57 per cent, respectively; d-tubocurarine at concentrations of 10^{-6} M, 10^{-5} M, 10^{-4} M, and 10^{-3} M inhibited ChAT activity by 0, 4, 12.5 and 29 per cent, respectively; whereas succinylcholine at concentrations of 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4} M activated ChAT activity by 8, 10, 1, and 2 per cent, respectively.

Key words

ENZYME: choline acetyltransferase;

NEUROMUSCULAR RELAXANTS: pancuronium, succinylcholine, d-tubocurarine, vecuronium.

From the Departments of *Anesthesiology and †Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee.

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Address correspondence and reprint requests to: Dr. J.R. Kambam, Department of Anesthesiology, Vanderbilt University Hospital, Nashville, Tennessee 37232.

Even though our present data demonstrated a significant dose-dependent inhibitory effect on ChAT activity by pancuronium, vecuronium and d-tubocurarine, it is unlikely that this inhibitory effect will contribute to the mechanism of action of NDMR. Our data, however, may suggest an additional mechanism for the phenomena of tetanic and train-of-four fades that are seen following the administration of nondepolarizing muscle relaxants.

Nous avons mesuré les effets de trois myorelaxants à action non-dépolarisante : le pancuronium, le vécuronium et la d-tubocurarine et de la succinylcholine (action dépolarisante) sur l'activité de la choline acétyltransférase (ChAT). Nous avons utilisé une mesure radiochimique de l'activité de la ChAT à partir d'enzyme purifié d'origine placentaire. Le pancuronium en concentration de 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M et 10^{-3} M diminuait l'activité de la ChAT de 3, 10, 15, 40 et 85 pour cent respectivement alors que le vécuronium à 10^{-6} M, 10^{-5} M, 10^{-4} M et 10^{-3} M la diminuait de 5, 10, 26 et 57 pour cent et la d-tubocurarine à 10^{-6} M, 10^{-5} M, 10^{-4} M et 10^{-3} M la réduisait de 0, 4, 12,5 et 29 pour cent. Par contre, la succinylcholine en concentration de 10^{-7} M, 10^{-6} M, 10^{-5} M et 10^{-4} M, augmentait l'activité de la ChAT de 8, 10, 1 et 2 pour cent respectivement. Il est toutefois peu probable que cette inhibition significative de la ChAT proportionnelle à la dose de pancuronium, de vécuronium et de d-tubocurarine soit à la base du mécanisme d'action des myorelaxants de type non-dépolarisant. Elle pourrait cependant contribuer à l'épuisement de la réponse à la stimulation tétonique ou en train-de-quatre qu'on observe avec ces agents.

It has long been known that the nondepolarizing muscle relaxants not only depress peak tension, but also produce

fade of responses to repeated stimulation, namely tetanic fade (TF) and train-of-four fade (TOFF).^{1,2} A desensitization block sometimes caused by the administration of succinylcholine is also associated with TF and TOFF. There have been conflicting data regarding the mechanism of tetanic and train-of-four fades.³⁻⁶ Investigators have ascribed the mechanisms responsible for these fade phenomena caused by nondepolarizing muscle relaxants to either a fall off in acetylcholine release during the stimulus or stimuli (prejunctional effect) or occlusion of postjunctional cholinergic receptor-operated ion channels (postjunctional effect).³⁻⁶

Acetylcholine is the principal chemical transmitter at the neuromuscular junction. It is now established that the enzyme choline acetyltransferase (ChAT, acetyl-CoA:choline-O-acetyltransferase) catalyzes the transfer of the acetyl moiety from acetyl CO-A to choline. This final step in the biosynthesis of acetylcholine involves the following reaction:

Certain drugs are known to decrease the synthesis of acetylcholine at the nerve terminal by inhibiting choline acetyltransferase (ChAT) activity.⁷⁻⁹ If the neuromuscular relaxants gain access to ChAT at the somatic nerve terminal, they may inhibit ChAT, decrease Ach synthesis, and thereby modify cholinergic transmission. Such an inhibition caused by neuromuscular blocking agents on ChAT activity could provide an additional mechanism for the mode of action as well as the phenomena of tetanic and train-of-four fades. Furthermore, to our knowledge, there is no information available on the effects of neuromuscular blocking agents used in anaesthesia practice on ChAT activity. Therefore, we studied the effects of pancuronium, vecuronium, d-tubocurarine, and succinylcholine on ChAT activity.

Methods

The ChAT activity was determined by a radiochemical method modified from those of Sastry and Henderson¹⁰ in which the ¹⁴C-acetyl groups from ¹⁴C-AcCoA were transferred to choline with potassium phosphate buffer (50 mM, pH 7.4) as the reaction medium. ¹⁴C-acetylcholine (¹⁴C-Ach) and coenzyme A are formed as reaction products. The reaction mixture containing reactants (¹⁴C-AcCoA, choline) and products (¹⁴C-Ach, coenzyme A) were passed through a column of anion exchange resin. ¹⁴C-AcCoA and ¹⁴C-acetate (if present as an impurity in trace amounts) were adsorbed by the anion exchange column. ¹⁴C-Ach formed by enzymatic synthesis passed through the column and was recovered quantitatively in the effluates. By determining the radioactivity

of effluates, ¹⁴C-Ach formed by the ChAT could be calculated. To study the effects of ChAT by pancuronium (10⁻⁷ to 10⁻³ M), vecuronium (10⁻⁶ to 10⁻³ M), d-tubocurarine (10⁻⁶ M to 10⁻³ M), and succinylcholine (10⁻⁷ to 10⁻⁴ M), ChAT activity was determined with or without the neuromuscular relaxant in the reaction medium. The assay for ChAT activity was developed as follows. The total volume of the reaction mixture was 0.3 ml in potassium phosphate buffer which was composed of three 0.1 ml aliquots added sequentially to a 75 × 10 mm test tube. The first aliquot consisted of 0.1 ml of buffer which brought the final concentrations of its components to 0.3 M sodium chloride, 2 × 10⁻⁴ M physostigmine sulfate, 0.02 M magnesium sulfate, 0.005 M choline iodide, and 5 × 10⁻⁵ M labelled ¹⁴C-AcCoA plus unlabeled AcCoA. The second aliquot of 0.1 ml buffer was reserved for dissolving various neuromuscular blocking compounds. The third aliquot of 0.1 ml consisted of the purified placental ChAT enzyme in the phosphate buffer containing sodium cyanide (0.02 M) and disodium ethylenediaminetetraacetate (Na₂ EDTA, 5 × 10⁻⁴ M). Sodium cyanide and Na₂ EDTA prevent the loss of ChAT activity.

The reaction was started by the addition of the third aliquot to the mixture of the first two aliquots, and the mixture was incubated for ten minutes at 37° C. The reaction was stopped by pouring the contents of the tube on to a column of Bio-Rad AG-1-X8 anion exchange resin (200–400 mesh, chloride form). Distilled water, 0.5 ml, was added to the empty incubation tube and poured over the column. Water was allowed to disappear from top of packing and this procedure was repeated four more times at five-minute intervals. The effluent was collected in scintillation vials to which 15 ml of aqueous counting scintillant was added. These vials were counted in a Beckman Model 3133-P liquid scintillation counter and the ¹⁴C-yield was correlated to Ach production. The ChAT activity was expressed as units. One unit of ChAT activity is equivalent to one nanomole of Ach formed · mg⁻¹ of enzyme · 10 min⁻¹.

Statistics

Linear regression analyses and correlation coefficients were calculated between log muscle relaxant drug concentrations and ChAT activities. The per cent inhibition of ChAT activity was plotted against log concentrations of pancuronium and vecuronium and the concentrations producing 50 per cent inhibition of ChAT activity (I₅₀) were determined.

Results

The inhibitory effects of pancuronium, vecuronium, d-tubocurarine, and succinylcholine on ChAT activity are

TABLE I Effect of pancuronium on choline acetyltransferase (ChAT) activity

Pancuronium concentration	ChAT activity units mean $\pm$ SD	% Inhibition of ChAT activity
Control (0)	335 $\pm$ 10	—
10 ⁻⁷ M	325 $\pm$ 12	3.0
10 ⁻⁶ M	302 $\pm$ 22	10.0
10 ⁻⁵ M	284 $\pm$ 12	15.0
10 ⁻⁴ M	200 $\pm$ 7	40.0
10 ⁻³ M	57 $\pm$ 7	85.0

n = 6.

TABLE II Effect of vecuronium on choline acetyltransferase (ChAT) activity

Vecuronium concentration	ChAT activity units mean $\pm$ SD	% Inhibition of ChAT activity
Control (0)	359 $\pm$ 2	—
10 ⁻⁶ M	341 $\pm$ 2	5.0
10 ⁻⁵ M	321 $\pm$ 5	11.0
10 ⁻⁴ M	261 $\pm$ 5	28.0
10 ⁻³ M	142 $\pm$ 5	61.0

n = 6.

shown in Tables I, II, III, and IV, respectively. Table V contains the mean values of slopes, intercepts, and correlation coefficients with corresponding *P* values. Pancuronium, vecuronium, and to a lesser extent d-tubocurarine inhibited ChAT activity significantly (*P* < 0.01). Unlike the nondepolarizing muscle relaxants succinylcholine, a depolarizing muscle relaxant, slightly stimulated ChAT activity (*P* < 0.05). The mean log concentrations necessary to inhibit 50 per cent of ChAT activity (*I*₅₀) were 5.5×10^{-4} M for pancuronium and 6.2×10^{-4} M for vecuronium.

Discussion

Our present data demonstrated that pancuronium, vecuronium and d-tubocurarine inhibit acetylcholine synthesis in a dose-dependent manner by inhibiting ChAT activity. These depressant effects of NDMR on ChAT activity are too small (5–15 per cent decrease) at clinical concentrations (10⁻⁶ to 10⁻⁵ M) to contribute to the neuromuscular blocking effect, but prolonged small depressant effect on ChAT activity may offset the balance between synthesis and release of Ach and thus may manifest as tetanic and train-of-four fades. It has previously been shown that during the course of a neuromuscular block in humans, tetanic fade is slower to develop and TOFF is more pronounced during recovery than during the onset of the

TABLE III Effect of d-tubocurarine (dTC) on choline acetyltransferase (ChAT) activity

dTC concentration	ChAT activity units mean $\pm$ SD	% Inhibition of ChAT activity
Control (0)	227 $\pm$ 15	—
10 ⁻⁶ M	225 $\pm$ 15	1
10 ⁻⁵ M	218 $\pm$ 12	4
10 ⁻⁴ M	201 $\pm$ 15	12.5
10 ⁻³ M	162 $\pm$ 15	29.0

n = 6.

TABLE IV Effect of succinylcholine (SCh) on choline acetyltransferase (ChAT) activity

Succinylcholine concentration	ChAT activity units mean $\pm$ SD	% Activation of ChAT activity
Control (0)	146 $\pm$ 7	—
10 ⁻⁷ M	158 $\pm$ 2	8
10 ⁻⁶ M	161 $\pm$ 2	10
10 ⁻⁵ M	148 $\pm$ 4	1
10 ⁻⁴ M	150 $\pm$ 7	2

n = 5.

block.¹¹ Unlike the nondepolarizing muscle relaxants, our data show that the depolarizing muscle relaxant, succinylcholine, slightly activates Ach synthesis in a dose-independent manner. Therefore, our data failed to provide a mechanism for desensitization block caused by succinylcholine. Our data on succinylcholine contradicts the data published earlier.¹² Previously it has been reported that succinylcholine (10⁻⁴ M) inhibits about 20 per cent of ChAT activity. Differences in source, purity of the enzyme, and methodology may account for this discrepancy.

There is evidence that d-tubocurarine and related drugs impair Ach release at the nerve terminal, especially during repetitive nerve activity.^{13–14} There is also evi-

TABLE V Linear regression analyses between log concentrations of pancuronium, vecuronium, d-tubocurarine, and succinylcholine and choline acetyltransferase (ChAT) activities. Mean values of intercept, slope, and correlation coefficients (*r*) with corresponding *P* values are shown

	Intercept	Slope	<i>r</i>	<i>P</i>
Pancuronium (<i>n</i> = 6)	-2.13	-0.001	0.84	<0.01
Vecuronium (<i>n</i> = 6)	-0.86	-0.001	0.95	<0.01
d-Tubocurarine (<i>n</i> = 6)	3.73	-0.005	0.90	<0.01
Succinylcholine (<i>n</i> = 5)	23.20	-0.020	0.71	<0.05

dence that d-tubocurarine impairs mobilization of Ach within nerve endings.¹³ Investigators have also shown that in the presence of pancuronium and vecuronium there is a decrease in quantal content of Ach in the nerve terminal.¹⁵⁻¹⁶

Even though muscle relaxants are said to be completely ionized at body pH they do cross cell membranes.¹⁷⁻²⁰ Notable concentrations have been found in the cerebrospinal fluid (CSF) as well as in the umbilical cord blood within a short time after the intravenous administration of muscle relaxant.^{17,19,20} Pancuronium, but not other neuromuscular relaxants, is known to decrease the anaesthetic concentration requirement for halothane.²¹

Choline acetyl transferase, the principal enzyme responsible for the synthesis of acetylcholine, is a slowly acting enzyme when compared with catabolizing enzymes of Ach such as acetylcholinesterase and plasma cholinesterase. However, there are special storage mechanisms for Ach at the nerve terminal to meet the demands. In spite of the large reserves of Ach at the nerve endings, tetanic fade can be detected even in the absence of neuromuscular blocker if strong enough stimuli are applied.²² The margin of safety of neuromuscular transmission is such that a normal twitch response to a single stimulus applied to a motor nerve can be elicited even when 75-80 per cent of the postsynaptic receptors are occluded by competitive neuromuscular blockers.²³ However, TOFF and TF can be demonstrated when more than 80 per cent and 50 per cent of the receptors are occluded by a competitive neuromuscular blocker, respectively.²³ In addition, a small decrease in Ach reserves may contribute to the phenomena of tetanic and train-of-four fades, especially in the presence of Ach antagonists like nondepolarizing muscle relaxants.

In conclusion, our data demonstrated that pancuronium, vecuronium, and to a lesser extent d-tubocurarine possess a dose-dependent inhibitory effect on choline acetyltransferase activity, whereas succinylcholine, a depolarizing muscle relaxant, has a slight stimulatory effect. Our data suggest an additional mechanism for the phenomena of tetanic and train-of-four fades that are seen following the administration of nondepolarizing muscle relaxants.

References

- 1 Paton WDM, Zaimis EJ. The action of d-tubocurarine and of decamethonium on respiratory and other muscles in the cat. *J Physiol (Lond)* 1951; 112: 311-31.
- 2 Ali HH, Utting JE, Gray TC. Quantitative assessment of residual antidepolarizing block. *Br J Anaesth* 1971; 43: 478-85.
- 3 Adams PR. A model for the procaine end-plate current. *J Physiol (Lond)* 1975; 246: 62-3.
- 4 Adams PR. Drug blockade of open end-plate channels. *J Physiol (Lond)* 1976; 260: 531-52.
- 5 Magleby KL, Pallotta BS, Terrar DA. The effect of $\pm$ tubocurarine on neuromuscular transmission during repetitive stimulation in the rat, mouse, and frog. *J Physiol (Lond)* 1981; 312: 97-113.
- 6 Katz B, Miledi R. Further observations on acetylcholine noise. *Nature* 1971; 232: 124-6.
- 7 deBailbain V, Haarstad VB, White ED. Inhibition of brain choline acetylase by acetylsecohemicholinium. *Pharmacologist* 1968; 10: 223.
- 8 Domino EF, Mohrman ME, Wilson AE, Haarstad VB. Acetylsecohemicholinium-3, a new choline acetyltransferase inhibitor useful in neuropharmacological studies. *Neuropharmacology* 1973; 12: 549-61.
- 9 Rowell PP, Chaturvedi AK, Sastry BVR. (2 Benzoylethyl) ethyltrimethylammonium chloride: a new, selective and stable inhibitor of human placental choline acetyltransferase. *J Pharmacol Exp Ther* 1978; 205: 624-34.
- 10 Henderson GE, Sastry BVR. Human placental choline acetyltransferase: nature and molecular aspects of the inhibition by iodo- and bromoacetylcholines. *Biochem Pharmacol* 1978; 27: 1331-40.
- 11 Sugai N, Hughes R, Payne JP. Sequential changes in the fade of tetanic tension after the administration of tubocurarine in anesthetized man. *Br J Anaesth* 1976; 48: 535-9.
- 12 Reisberg RB. Properties and biological significance of choline acetylase. *Yale J Biol Med* 1957; 29: 403-35.
- 13 Lilleheil G, Naess K. A presynaptic effect of d-tubocurarine in the neuromuscular junction. *Acta Physiol Scand* 1961; 52: 120-36.
- 14 Blaber LC. The prejunctional actions of some non-depolarizing blocking drugs. *Br J Pharmacol* 1973; 47: 109-16.
- 15 Su PC, Su WL, Rosen AD. Pre- and postsynaptic effects of pancuronium at the neuromuscular junction of the mouse. *Anesthesiology* 1979; 50: 199-204.
- 16 Torda TA, Kiloh N. Myoneural actions of Org NC 45. *Br J Anaesth* 1982; 54: 1217-21.
- 17 Matteo RS, Pua EK, Khambatta HJ, Spector S. Cerebrospinal fluid levels of d-tubocurarine in man. *Anesthesiology* 1977; 46: 396-9.
- 18 Munson ES, Wagman IH. Elevation of lidocaine seizure threshold by gallamine in Rhesus monkeys. *Arch Neurol* 1973; 28: 329-33.
- 19 Dailey PA, Fisher DM, Shnider SM. Pharmacokinetics, placental transfer, and neonatal effects of vecuronium and pancuronium administered during cesarean section. *Anesthesiology* 1984; 60: 569-74.

- 20 *Demetriou M, DePoix JP, Diakite B.* Placental transfer of Org NC 45 in women undergoing caesarean section. *Br J Anaesth* 1982; 54: 643-5.
- 21 *Forbes AR, Cohen NH, Eger EI II.* Pancuronium reduces halothane requirement in man. *Anesth Analg* 1979; 58: 497-9.
- 22 *Bowman WC.* Prejunctional and postjunctional cholinergic receptors at the neuromuscular junction. *Anesth Analg* 1980; 59: 935-43.
- 23 *Waud BE, Waud DR.* Physiology and pharmacology of neuromuscular blocking agents: *In:* Katz RL (Ed.). *Muscle Relaxants*. New York; American Elsevier, 1975, 1-58.