Lack of effects of d-tubocurarine and pancuronium on the slow action potential of the guinea pig papillary muscle

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Inotropic effects of non-depolarizing muscle relaxants were examined with guinea pig ventricular papillary muscle depolarized to -47 mV in high K Ba-Tyrode solution. Field stimulation of 0.1 Hz elicited the slow action potential, a measure of the calcium current. The amplitude, the duration at 0 mV level and dV/dt of the action potential were monitored together with the contractile tension. Amelizol ($3 \text{ mg}\cdot\text{ml}^{-1}$ d-tubocurarine (d-tc) and $5 \text{ mg}\cdot\text{ml}^{-1}$ chlorobutanol) depressed the four functions in a dose-dependent manner, while crystalline d-tc did not. Chlorobutanol (the antimicrobial preservative) had the same effects as Amelizol. Neither Mioblock ($2 \text{ mg}\cdot\text{ml}^{-1}$ pancuronium and unpublished preservative) nor crystalline pancuronium altered the functions. These findings suggest that the negative inotropic effect of Amelizol is not due to d-tc but to chlorobutanol, which may exert its effect by depressing the calcium current. The lack of change in the slow action potential seen with pancuronium may indicate no direct effect on the calcium current, thereby further suggesting absent direct beta-adrenomimetic action of this agent.

Key words

NEUROMUSCULAR RELAXANTS: d-tubocurarine, pancuronium; MUSCLE, CARDIAC: slow action potential; ANTIMICROBIALS: chlorobutanol.

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The importance of non-depolarizing muscle relaxants in clinical anaesthesia is widely recognized. Investigators have reported adverse effects of some of these agents. Intravenous administration of various commercial preparations of d-tubocurarine (d-tc) may induce arterial hypotension¹ which was ascribed to a reduction in the peripheral vascular resistance² caused by an increase in the plasma histamine level³ and/or ganglionic blockade.^{4,5} The commercial preparations were shown to have a negative inotropic effect on the myocardium *in vitro*, and this effect was attributed to their preservatives.^{6,7} Carrier and Murphy further showed that elevation of the calcium ion concentration in the perfusate reversed the negative inotropic effect of Abbott vehicle 3386 and benzyl alcohol (a bacteriostatic preservative in Squibb's tubocurarine chloride). They did not, however, monitor the electrical phenomena of the cardiac muscle. Therefore it was not possible to elucidate where these agents exerted the effects in the sequence of excitation-contraction coupling.

In the present study, experiments were designed to assess the effects of a commercial preparation Amelizol ($3 \text{ mg}\cdot\text{ml}^{-1}$ d-tc and $5 \text{ mg}\cdot\text{ml}^{-1}$ chlorobutanol) on the slow inward current and the contractile tension by use of the slow action potential and the associated contraction of partially depolarized papillary muscle of guinea pig ventricle.

On the other hand we often observed tachycardia and hypertension after intravenous administration of Mioblock ($2 \text{ mg}\cdot\text{ml}^{-1}$ pancuronium and unpublished preservative). Tachycardia sometimes leads to serious hypotension in patients with valvular heart disease and may have adverse effects on the

ischaemic heart muscle by increasing oxygen demand. These cardioacceleratory effects have been studied by many investigators⁸⁻¹² and attributed to a direct action on the myocardium,¹³ a vagolytic action^{2,14,15} or an increase in catecholamine release.^{16,17}

The present *in vitro* experiments were designed to study the inotropic effects of a commercial preparation (Mioblock) and crystalline pancuronium. It was possible to clarify the direct effects of the agents on the slow action potential and the contractile tension, because the preparation was dissociated from control of the central nervous system and because catecholamine release from the nerve endings in the myocardium was minimized by use of low frequency stimulation and a partially depolarized preparation.

Methods

Guinea pigs (300–400 g) were killed by a blow on the head and the heart was quickly excised. Papillary muscles with a diameter of at most 1 mm were dissected from the right ventricle and mounted in a recording chamber of 1 mL volume, which was perfused at a constant rate of 5 mL·min⁻¹ with Tyrode solution of the following composition (mM): NaCl 137; KCl 2.2; CaCl₂ 1.8; MgCl₂ 1.1; NaH₂PO₄ 0.4; NaHCO₃ 12; glucose 5.5. The solution was aerated with a mixture of 95 per cent O₂ and five per cent CO₂ and maintained at 36.0 ± 0.5°C.

The valvular end of the muscle was connected to a linear force-displacement transducer (UL-2; Shinkoh, Japan) for isometric tension measurement. Membrane potentials were measured with 3M KCl-filled glass microelectrodes with a resistance of about 20 megohms. Potentials were led to an amplifier with high input impedance and negative capacitance adjustment (MEZ 7101; Nihon Kohden, Japan). Analog differentiation of the action potential (dV/dt) was obtained with an electronic differentiator with a time constant of 10 or 100 µsec. For stimulation, rectangular current pulses were applied between the Ag-AgCl plate electrode placed near the valvular end of the muscle and a small ring of Ag-AgCl wire closely encircling the basal segment of the muscle, as described by Ehara and Inazawa.¹⁸ The stimuli were delivered at a rate of 0.1 Hz via an isolator connected to an electronic stimulator (SS 102J and SEN 1101; Nihon Kohden,

Japan). The stimulus duration was 2 msec and the intensity was 1.5 times the threshold.

After equilibration for 30 to 60 minutes in normal Tyrode solution, the bath medium was replaced with high K Ba-Tyrode solution prepared by increasing KCl concentration to 22 mM and adding 0.2 mM BaCl₂. No correction for osmolarity was made. Drugs were applied after more than 30 minutes of equilibration in the high K Ba-Tyrode solution. Action potential, its dV/dt and contractile tension were monitored on an oscilloscope (5113; Tektronix, USA) and photographed. In some experiments a chart recorder (PMP 3004; Nihon Kohden, Japan) was also used. The action potential duration was measured at 0 mV level.

Drugs used were Amelizol (3 mg·ml⁻¹ d-tubocurarine chloride and 5 mg·ml⁻¹ chlorobutanol) (Yoshitomi, Japan), crystalline d-tubocurarine chloride (Nakarai, Japan), chlorobutanol (Nakarai, Japan), Mioblock (2 mg·ml⁻¹ pancuronium bromide and unpublished preservative) (Organon, Holland) and crystalline pancuronium bromide (Organon, Holland). All data considered were those from experiments where a single impalement of a cell was maintained throughout. All parameters were measured at about 20 minutes after application of the drugs. Recovery of about 90 per cent was achieved by washing the preparation for 30 minutes. Data were analyzed using Student's t-test for paired data.

Results

The resting membrane potential of the papillary muscle in normal Tyrode solution was -96.2 ± 0.67 mV (mean ± SEM) (n = 72). It was depolarized to -47 ± 0.67 mV (mean ± SEM) (n = 64) in high K Ba-Tyrode solution. The slow action potential and the contractile tension of the muscle were elicited by increasing the stimulus intensity (Figure 1 a, b). Low concentrations of Amelizol did not affect the parameters significantly while higher concentrations markedly depressed the slow action potential and the contractile tension, without affecting the resting membrane potential. The depression was completely reversed when Ca ion concentration of the perfusate was doubled (Figure 1 c, d). Similar results were obtained with four additional preparations. Figure 2 illustrates the effects of various concentrations of Amelizol on the parameters. Reduction in the action potential duration, maximum

FIGURE 1 Depression of the slow action potential and the contractile tension by Amelizol and reversal by elevation of calcium ion concentration. (a) Action potential, its dV/dt and contractile tension of guinea pig papillary muscle in normal Tyrode solution. (b) The slow action potential, its dV/dt and the contractile tension in high K Ba-Tyrode solution. The dV/dt reference line indicates 0 mV level for the action potential. (c) Depression of the parameters by Amelizol ($40 \text{ mg} \cdot \text{L}^{-1}$). (d) Reversal of the depression by elevation of calcium ion concentration (1.8 mM to 3.6 mM) of the perfusate. (e) Recovery after washing with high K Ba-Tyrode solution for 30 min. Calibrations for (b) apply also for (c), (d) and (e).

dV/dt and the contractile tension was already significant with $20 \text{ mg} \cdot \text{L}^{-1}$ Amelizol. All the parameters were reduced, in a concentration dependent manner.

Crystalline d-tc did not affect the slow action potential or the contractile tension, even at the highest concentration used ($40 \text{ mg} \cdot \text{L}^{-1}$) (Figure 3), while this concentration of Amelizol greatly reduced all the parameters (Figure 2). Chlorobutanol, the antimicrobial preservative in Amelizol, decreased the parameters significantly, in the same manner as Amelizol (Figure 4). As Amelizol contains $3 \text{ mg} \cdot \text{ml}^{-1}$ of d-tc and $5 \text{ mg} \cdot \text{ml}^{-1}$ of chlorobutanol, the concentration of chlorobutanol used in Figure 4 roughly corresponds to that contained in the Amelizol experiment (Figure 2). The depression by chlorobutanol ($50 \text{ mg} \cdot \text{L}^{-1}$) was also reversed in all of six experiments by elevation of calcium ion concentration in the perfusate.

Mioblock did not alter either the slow action potential or the associated contractile tension. Figure 5 shows the per cent change of the parameters from the control condition. The parameters were not affected significantly even at the highest concentration used ($20 \text{ mg} \cdot \text{L}^{-1}$). A high concentration of crystalline pancuronium bromide ($20 \text{ mg} \cdot \text{L}^{-1}$) did not affect the parameters ($n = 4$).

Discussion

To elucidate the effects of drugs on the myocardium, it is important to study the pharmacologic actions on the ionic mechanisms of the action potential. In particular, with regard to inotropic effects, changes in the membrane calcium current have to be examined. Calcium ions which flow into the heart muscle cell during the action potential appear to release calcium ions from an intracellular storage site through a calcium induced calcium

FIGURE 2 Effects of various concentrations of Amelizol on the slow action potential and the contractile tension. Amelizol reduced the action potential amplitude (APA), action potential duration at 0 mV level (APD_{0mV}), maximum upstroke velocity of action potential (max dV/dt) and contractile tension, in a dose dependent manner. Significant changes from the control value ($p < 0.05$) are indicated by asterisks. Number of experiments is shown in parentheses.

FIGURE 3 Effects of crystalline d-tubocurarine on the slow action potential and the contractile tension. Even a high dose of the agent (40 mg·L⁻¹) did not affect the parameters. No significant change was observed with each concentration. Number of the experiments is shown in parentheses.

FIGURE 4 Effects of chlorobutanol on the slow action potential and the contractile tension. Chlorobutanol depressed the slow action potential and the contractile tension, in a dose dependent manner. Significant changes from the control value ($p < 0.05$) are indicated by asterisks. The effect was similar to that of Amelizol. Number of experiments is shown in parentheses.

release mechanism.¹⁹ Furthermore, the calcium current itself provides calcium ions to the intracellular storage site.

The slow action potential of the partially depolarized myocardium is known to be mediated by the slow channel and is useful for qualitative studies on the slow inward current which consists mainly of the calcium current.²⁰ There are several reports on the inotropic effects of anaesthetics using this method.²¹⁻²³ In the present study, the slow action potential of 22 mM K-depolarized guinea pig papillary muscle was facilitated by 0.2 mM BaCl₂ with reduced membrane shunt resistance, as described by Ehara and Inazawa.¹⁸ At the membrane potential of about -47 mV, the fast sodium current was almost completely inactivated, whereas the slow calcium current was little affected.²⁴ The low concentration of Ba does not contribute appreciably to the upstroke velocity of the slow action potential, with normal calcium concentrations. The maximum rate of rise of the membrane action potential is considered to be a measure of the membrane current, in the present experiments, namely, the calcium inward current.

Carrier and Murphy⁷ and Dowdy *et al.*⁶ reported that the negative inotropic effect of the commercial

FIGURE 5 Effects of Mioblock on the slow action potential and the contractile tension. The drug did not affect the action potential or the contractile tension. No changes were significant at any concentration. Number of experiments is shown in parentheses.

preparations of d-tc *in vitro* was due to their preservatives. In the present study, we found that crystalline d-tc did not affect the slow action potential and that chlorobutanol significantly depressed the slow action potential and the associated contractile tension. These events were effectively reversed by elevation of calcium ion concentration in the perfusate. Our findings indicate that chlorobutanol exerts its negative inotropic effect via reduction in the calcium inward current, at least to some extent. Wingard and Cook reported that the serum concentration of d-tc was $9.6 \text{ mg}\cdot\text{L}^{-1}$ immediately after and $3.86 \text{ mg}\cdot\text{L}^{-1}$ two minutes after the intravenous injection of $0.3 \text{ mg}\cdot\text{kg}^{-1}$ in man, with estimation of the initial apparent volume of distribution to be 3 to 3.6 liters.²⁵ Higher concentrations of the drug may reach the myocardium at the very early circulation due to a smaller distribution volume for the drug. If the concentration of chlorobutanol declines in the same manner as d-tc, it may reach a level of more than $30 \text{ mg}\cdot\text{L}^{-1}$ in the myocardial circulation, high enough to contribute to arterial hypotension. A concentration of $60 \text{ mg}\cdot\text{L}^{-1}$ may be attained in the case of an overdose in clinical anaesthesia.

Stoelting reported that an intravenous injection of 10 ml of Lilly's diluent (sodium chloride $7 \text{ mg}\cdot\text{ml}^{-1}$; sodium bisulfite $1 \text{ mg}\cdot\text{ml}^{-1}$; chlorobutanol $5 \text{ mg}\cdot\text{ml}^{-1}$) in halothane-nitrous oxide-oxygen anaesthetized men did not alter the mean arterial

pressure significantly while Lilly's d-tc did.²⁶ The discrepancy may be due to differences in species and preparations used. It may therefore be concluded that the negative inotropic effect of Amelizol (d-tubocurarine-chlorobutanol) is partly due to the depressant effect of the preservative (chlorobutanol) on the calcium inward current.

The mechanism of effects of pancuronium on the circulatory system is controversial. Muscarinic receptor blockade, beta-adrenergic stimulation and a direct positive inotropic effect on the myocardium have been reported. Iwatsuki *et al.*²⁷ reported a positive inotropic effect of the drug on isolated canine ventricular trabeculae. They attributed the effect to a beta-adrenomimetic action, showing elimination of the effect in the presence of 10^{-6} M propranolol. In their experiments, the drug may have increased catecholamine release from sympathetic nerve terminals in the cardiac muscle, as indicated by Domenech *et al.*,¹⁷ because electrical field stimulation can release catecholamines from the nerve endings.²⁸ In K-depolarized preparations in the present experiments, catecholamines were likely not released in any considerable amount because the stimulation frequency was low and because of the relative incapability of nerve fibres to generate action potentials in the depolarized state. Therefore, it is likely that the previously reported positive inotropic effect of pancuronium was mediated by increased catecholamine release from the nerve endings.

Seed and Chamberlain reported a direct inotropic effect of the drug on dog heart *in vivo* but it was not great enough to increase the stroke volume or the mean arterial pressure.¹³ Agoston *et al.*²⁹ showed that the serum concentration of pancuronium two minutes after injection of $0.1 \text{ mg}\cdot\text{kg}^{-1}$ was about $1.0 \text{ mg}\cdot\text{L}^{-1}$ and Iwatsuki *et al.* calculated the concentration immediately after injection to be about $2.5 \text{ mg}\cdot\text{L}^{-1}$, using Wingard's equation.²⁷ Even taking into account a smaller initial distribution volume, our present findings show that clinical concentrations of pancuronium neither increase the calcium inward current nor exert a direct positive inotropic effect on the heart. Tachycardia and hypertension immediately after injection of the drug may be attributed to muscarinic blockade and/or increased release of catecholamines from the sympathetic nerve terminals.

Acknowledgements

We thank M. Ohara for comments on the manuscript. Crystalline pancuronium bromide was kindly provided by Organon (Holland).

References

- 1 Stoelting RK. The hemodynamic effects of pancuronium and d-tubocurarine in anesthetized patients. *Anesthesiology* 1972; 36: 612-5.
- 2 Coleman AJ, Downing JW, Leary WP, Moyes DG, Styles M. The immediate cardiovascular effects of pancuronium, alcuronium and tubocurarine in man. *Anaesthesia* 1972; 27: 415-22.
- 3 Iwatsuki K, Yusa T, Kataoka Y. Effects of muscle relaxants on ventricular contractile force in dogs. *Tohoku J Exp Med* 1965; 86: 9-18.
- 4 McCullough LS, Reier CE, Delaunois AL, Gardier R, Hamelberg W. The effects of d-tubocurarine on spontaneous post-ganglionic sympathetic activity and histamine release. *Anesthesiology* 1970; 33: 328-34.
- 5 Hughes R, Chapple DJ. Effects of non-depolarizing neuromuscular blocking agents on peripheral autonomic mechanisms in cats. *Br J Anaesth* 1976; 48: 59-67.
- 6 Dowdy EG, Holland WC, Yamanaka I, Kaya K. Cardioactive properties of d-tubocurarine with and without preservatives. *Anesthesiology* 1971; 34: 256-61.
- 7 Carrier O Jr, Murphy JC. The effects of d-tubocurarine and its commercial vehicles on cardiac function. *Anesthesiology* 1970; 33: 627-34.
- 8 Loh L. The cardiovascular effects of pancuronium bromide. *Anaesthesia* 1970; 25: 356-63.
- 9 McIntyre JWR, Gain EA. Initial experience during the clinical use of pancuronium bromide. *Anesth Analg* 1971; 50: 813-8.
- 10 Kelman GR, Kennedy BR. Cardiovascular effects of pancuronium in man. *Br J Anaesth* 1971; 43: 335-8.
- 11 Gertel M, Fox GS, Rabow FI, Graham DH. The cardiovascular effects of pancuronium during halothane anaesthesia. *Can Anaesth Soc J* 1972; 19: 599-606.
- 12 Edwards RP, Miller RD, Roizen MF *et al.* Cardiac responses to imipramine and pancuronium during anaesthesia with halothane or enflurane. *Anesthesiology* 1979; 50: 421-5.
- 13 Seed RF, Chamberlain JH. Myocardial stimulation by pancuronium bromide. *Br J Anaesth* 1977; 49: 401-7.
- 14 Saxena PR, Bonta IL. Mechanism of selective vagolytic action of pancuronium bromide. Specific blockade of cardiac muscarinic receptors. *Eur J Pharmacol* 1970; 11: 332-41.
- 15 Duke PC, Fung H, Gartner J. The myocardial effects of pancuronium. *Can Anaesth Soc J* 1975; 22: 680-6.
- 16 Nana A, Cardan E, Domokos M. Blood catecholamine changes after pancuronium. *Acta Anaesthesiol Scand* 1973; 17: 83-7.
- 17 Domenech JS, Garcia RC, Sasiain JMR, Loyola AQ, Oroz JS. Pancuronium bromide: An indirect sympathomimetic agent. *Br J Anaesth* 1976; 48: 1143-8.
- 18 Ehara T, Inazawa M. Calcium-dependent slow action potentials in potassium-depolarized guinea-pig ventricular myocardium enhanced by barium ions. *Naunyn-Schmiedeberg's Arch Pharmacol* 1980; 315: 47-54.
- 19 Endo M. Mechanism of calcium-induced calcium release in the SR membrane. In: Ohnishi ST, Endo M, eds. *The mechanism of gated calcium transport across biological membranes*. New York: Academic Press, 1981: 257-64.
- 20 Schneider JA, Sperelakis N. Slow Ca^{2+} and Na^{+} responses induced by isoproterenol and methylxanthines in isolated perfused guinea pig hearts exposed to elevated K^{+} . *J Mol Cell Cardiol* 1975; 7: 249-73.
- 21 Ikemoto Y. Reduction by thiopental of the slow-channel-mediated action potential of canine papillary muscle. *Pflüger's Arch* 1977; 372: 285-6.
- 22 Lynch C, Vogel S, Sperelakis N. Halothane depression of myocardial slow action potentials. *Anesthesiology* 1981; 55: 360-8.
- 23 Lynch C, Vogel S, Pratila MG, Sperelakis N. Enflurane depression of myocardial slow action potentials. *J Pharmacol Exp Ther* 1982; 222: 405-9.
- 24 Reuter H. Divalent cations as charge carriers in excitable membranes. In: Barker PF, Reuter H, eds. *Calcium movement in excitable cells*. New York: Pergamon Press, 1975: 55-97.
- 25 Wingard LB, Cook DR. Pharmacodynamics of tubocurarine in humans. *Br J Anaesth* 1976; 48: 839-45.
- 26 Stoelting RK. Blood-pressure responses to

- d-tubocurarine and its preservatives in anesthetized patients. *Anesthesiology* 1971; 35: 315-7.
- 27 Iwatsuki N, Hashimoto Y, Amaha K, Obara S, Iwatsuki K. Inotropic effects of non-depolarizing muscle relaxants in isolated canine heart muscle. *Anesth Analg* 1980; 59: 717-21.
- 28 Blinks JR. Field stimulation as a means of effecting the graded release of autonomic transmitters in isolated heart muscle. *J Pharmacol Exp Ther* 1966; 151: 221-35.
- 29 Agoston S, Crul JF, Kerston UW, Scaf AHJ. Relationship of serum concentration of pancuronium to its neuromuscular activity in man. *Anesthesiology* 1977; 47: 509-12.

Résumé

Les effets inotropes des relaxants musculaires non dépolarisants ont été étudiés avec des muscles papillaires ventriculaires de cobayes dépolarisés à -47 mV dans un bain de solution à haute concentration de potassium-barium-Tyrode. Un champ de stimulation de 0.1 Hz a provoqué un potentiel d'action lent représentant une mesure du courant calcique. L'amplitude ainsi que la durée du potentiel d'action à 0 mV et le dV/dt ont été étudiées en même temps que la force contractile. L'amelizol (3 mg·ml⁻¹ d-tubocurarine (d-tc) et 5 mg·ml⁻¹ chlorobutanol) a provoqué une dépression des paramètres dépendamment de la dose administrée alors que la d-tc cristalline n'a provoqué aucun changement. Le chlorobutanol (le préservatif bactériostatique) a eu les mêmes effets que l'amelizol. Ni le mioblock (2 mg·ml⁻¹ pancuronium et préservatif non publié) ni le pancuronium cristalline n'ont altéré les paramètres. Ces résultats suggèrent que les effets inotropes négatifs de l'amelizol ne sont pas dus à la d-tc mais au chlorobutanol qui exerce ses effets en déprimant les courants calciques. L'absence de changement dans le potentiel d'action lent observé avec le pancuronium peut indiquer qu'il n'a aucun effet direct sur les courants calciques suggérant ainsi l'absence d'effet direct bêta-adrinomimétique de cet agent.