Inhibition by vecuronium of carbachol-induced influx of $^{22}$Na$^+$, $^{45}$Ca$^{2+}$ and secretion of catecholamines in cultured bovine adrenal medullary cells

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Summary. In cultured bovine adrenal medullary cells, vecuronium, pancuronium and D-tubocurarine reduced carbachol-induced $^{45}$Ca$^{2+}$ influx and catecholamine secretion by inhibiting $^{22}$Na$^+$ influx via nicotinic receptor-ion channel complex with IC$_{50}$ values of 0.43, 7.6 and 3.9 mmol/l, respectively. IC$_{50}$ values of pancuronium and D-tubocurarine observed in adrenal medulla were one order of magnitude higher than the plasma concentrations of these muscle relaxants reported to produce 50% neuromuscular blockade, while IC$_{50}$ of vecuronium was quite close between adrenal medulla and skeletal muscle.

Key words: Adrenal medulla — Catecholamine secretion — Nicotinic receptor-ion channel complex — Sodium influx — Vecuronium

Introduction

In experimental animals (Durant et al. 1979) and clinical evaluations (Agoston et al. 1980; Fahey et al. 1981), vecuronium, a non-depolarizing neuromuscular blocking agent, has been reported to exhibit no deleterious effects on cardiovascular and autonomic nervous systems (see review Higlgenberg 1983). However, there is an increasing number of anesthetists who observed that vecuronium caused asystole, severe bradycardia and hypotension even in patients without cardiovascular disorders, particularly at the time of vagal stimulation during surgical operation (Kirkwood and Duckworth 1983; Pryne and Edwards 1985). It has not been defined whether vecuronium is a primary causative agent for these untoward effects or whether hemodynamic responses to vagal stimulation are unopposed due to the inability of vecuronium to inhibit cardiac muscarinic receptors (Inoue et al. 1988). Although circulating catecholamines in plasma contribute to the change in heart rate and systemic blood pressure, previous studies have not examined the effects of vecuronium on the secretion of catecholamines.

Adrenal medullary cells, embryologically analogous to sympathetic ganglia, generate action potentials (Kido et al. 1982) and secrete catecholamines into systemic circulation. In cultured bovine adrenal medullary cells, our previous studies suggest that carbachol-induced influx of Na$^+$ via nicotinic receptor-associated ion channels and subsequent increase in cellular Na$^+$ leads to gated voltage-dependent Ca$^{2+}$ channels and cause catecholamine secretion (Wada et al. 1985, 1986). In the present study, we compared the potencies of vecuronium for carbachol-induced influx of $^{22}$Na$^+$, $^{45}$Ca$^{2+}$ and secretion of catecholamines with those of D-tubocurarine and pancuronium in cultured bovine adrenal medullary cells.

Materials and methods

Materials. Oxygenated Krebs-Ringer phosphate (KRP) buffer was composed of (mmol/l): NaCl 154, KCl 5.6, MgSO$_4$ 1.1, CaCl$_2$ 2.2, NaH$_2$PO$_4$ 0.85, Na$_2$HPO$_4$ 2.15, glucose 10 and 0.5% bovine serum albumin, pH 7.4.

Drugs. Eagle’s minimum essential medium, Nissui Seiyaku (Tokyo, Japan); calf serum, D-tubocurarine, Nakarai Chemicals (Kyoto, Japan); carbachol, collagenase, Sigma (St. Louis, MO, USA); hexamethonium, Tokyo Kasei (Tokyo, Japan). Vecuronium bromide and pancuronium bromide were kind gift from Sankyo (Tokyo, Japan). $^{22}$NaCl (6–17 Ci/mmol) was from New England Nuclear (Boston, MA, USA). $^{45}$CaCl$_2$ (0.5–2 Ci/mmol) was from Amersham International (Amersham, UK).

Isolation of adrenal medullary cells and primary culture. Isolated adrenal medullary cells were obtained by collagenase digestion of the slices of bovine adrenal medulla (Wada et al. 1985, 1986). Cells were suspended in Eagle’s minimum essential medium containing 10% calf serum, antibiotics and cultured (4 × 10$^6$ cells/dish, Falcon 35 mm) at 37°C in 5% CO$_2$-95% air (Wada et al. 1985, 1986). Cells (4 × 10$^6$) contained 49.2 ± 8.3 μg (n = 21) of catecholamines as adrenaline plus noradrenaline.

Secretion of catecholamines and influx of $^{45}$Ca$^{2+}$. Secretion of catecholamines and influx of $^{45}$Ca$^{2+}$ were measured simultaneously (Wada et al. 1985, 1986). Briefly, cells were incubated at 37°C for 1 min in 1 ml KRP buffer containing 2 μCi $^{45}$CaCl$_2$ with or without carbachol and various concentrations of vecuronium, D-tubocurarine and pancuronium. Reaction was terminated by adding hexamethonium (1 mmol/l), after which the medium was immediately transferred to a test tube and the cells were washed 4 times with ice-cold Ca$^{2+}$-free KRP buffer.

Catecholamines secreted into the medium were adsorbed to aluminium hydroxide and estimated by the ethylene-diamine condensation method (Wada et al. 1985, 1986).
Fig. 1. Concentration-inhibition curves of vecuronium, \(\alpha\)-tubocurarine and pancuronium for carbachol-induced \(\text{\textsuperscript{22}}\text{Na}^+\) influx. Cells (4 \(\times\) 10\(^6\)) were incubated at 37°C for 1 min with 2 \(\mu\text{Ci} \text{\textsuperscript{22}}\text{NaCl}\) in the absence or presence of carbachol (300 \(\mu\text{mol/l}\)) and various concentrations of vecuronium (\(\bullet\)), \(\alpha\)-tubocurarine (\(\circ\)) and pancuronium (\(\triangle\)). Values at 37°C in the absence of carbachol were subtracted. 100% on the ordinate represents carbachol-induced \(\text{Na}^+\) influx in the absence of muscle relaxants. Data shown are means \(\pm\) SD of 10 separate experiments.

Influx of \(\text{\textsuperscript{22}}\text{Na}^+\). Influx of \(\text{\textsuperscript{22}}\text{Na}^+\) was estimated in the same procedure as \(\text{\textsuperscript{45}}\text{Ca}^2+\) influx except that cells were incubated with 2 \(\mu\text{Ci} \text{\textsuperscript{22}}\text{NaCl}\) (Wada et al. 1985, 1986).

Statistical methods. All experiments were carried out in duplicate and each experiment was repeated at least 3 times. Data obtained are shown as means \(\pm\) SD.

Results

Effects of vecuronium, \(\alpha\)-tubocurarine and pancuronium on carbachol-induced influx of \(\text{\textsuperscript{22}}\text{Na}^+\), \(\text{\textsuperscript{45}}\text{Ca}^2+\) and secretion of catecholamines

Carbachol caused rapid and transient influx of \(\text{\textsuperscript{22}}\text{Na}^+\), \(\text{\textsuperscript{45}}\text{Ca}^2+\) and secretion of catecholamines and they reached the plateau at 1 min, as reported previously (Wada et al. 1985, 1986).

Carbachol (300 \(\mu\text{mol/l}\)) increased \(\text{Na}^+\) influx by 243.6 \(\pm\) 10.1 nmol/4 \(\times\) 10\(^6\) cells/min (\(n = 10\)) over the basal \(\text{Na}^+\) influx (10.1 \(\pm\) 0.7 nmol/4 \(\times\) 10\(^6\) cells/min) (\(n = 10\)). Carbachol (300 \(\mu\text{mol/l}\)) also increased \(\text{Ca}^2+\) influx and catecholamine secretion by 7.4 \(\pm\) 0.3 (\(n = 8\)) nmol and by 6.3 \(\pm\) 0.5 (\(n = 8\)) \(\mu\text{g}/4 \(\times\) 10\(^6\) cells/min over the basal \(\text{Ca}^2+\) influx (0.6 \(\pm\) 0.2 nmol/4 \(\times\) 10\(^6\) cells/min) (\(n = 8\)) and the basal secretion (0.7 \(\pm\) 0.2 \(\mu\text{g}/4 \(\times\) 10\(^6\) cells/min) (\(n = 8\)), respectively.

As shown in Fig. 1, vecuronium, at concentrations of 0.1 \(\mu\text{mol/l}\) and higher, inhibited carbachol-induced influx of \(\text{\textsuperscript{22}}\text{Na}^+\) with IC\(_{50}\) of 0.43 \(\mu\text{mol/l}\). \(\alpha\)-Tubocurarine and pancuronium suppressed carbachol-induced \(\text{\textsuperscript{22}}\text{Na}^+\) influx with IC\(_{50}\) values of 3.9 and 7.6 \(\mu\text{mol/l}\), respectively. In addition, vecuronium, \(\alpha\)-tubocurarine and pancuronium suppressed carbachol-induced influx of \(\text{\textsuperscript{45}}\text{Ca}^2+\) and secretion of catecholamines with concentration-inhibition curves similar to those for \(\text{\textsuperscript{22}}\text{Na}^+\) influx (Fig. 2).

Effects of vecuronium on carbachol-induced cellular responses evoked by various concentrations of carbachol

As shown in Fig. 3, the inhibitory effect of vecuronium (1 \(\mu\text{mol/l}\)) on carbachol-induced \(\text{\textsuperscript{22}}\text{Na}^+\) influx was not overcome by increasing concentrations of carbachol. Increasing concentrations of carbachol also failed to overcome the inhibition by vecuronium (1 \(\mu\text{mol/l}\)) of carbachol-induced \(\text{\textsuperscript{45}}\text{Ca}^2+\) influx and catecholamine secretion (Fig. 4).

Discussion

In the present study, vecuronium inhibited carbachol-induced influx of \(\text{\textsuperscript{22}}\text{Na}^+\), \(\text{\textsuperscript{45}}\text{Ca}^2+\) and secretion of catecholamines with similar potencies, as did \(\alpha\)-tubocurarine and...