Inhibitory Actions of Vecuronium Bromide on Acetylcholine and Glutamate Responses at the Frog and Crayfish Neuromuscular Junction

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Abstract—Effects of vecuronium bromide, an analog of pancuronium, on the cholinergic and glutamatergic neuromuscular junction were investigated. Vecuronium depressed the postsynaptic response of the frog end-plate at lower concentrations than $10^{-6}$ g/ml without affecting the presynaptic events. Vecuronium decreased the amplitude of the double ACh potential, but the second potential was more markedly reduced than the first. In analogy with d-tubocurarine, this suggests that vecuronium may act in part as an open channel blocker at the frog end-plate. Vecuronium depressed both the glutamate response and the excitatory junctional potential at the crayfish neuromuscular junction, although high concentrations were required. The drug increased the decay rate of extracellularly recorded excitatory junctional potentials at the crayfish neuromuscular junction. The reduction of the crayfish synaptic response caused by vecuronium can be explained by the open channel blocking action at this functional site. The problem that cholinergic antagonists possess a property of channel blocking at the other transmitter system was discussed.

Vecuronium is an analog of pancuronium, but is not the bis-quaternary ammonium, one quarternary moiety of pancuronium being replaced by a tertiary amine (Fig. 1). Although it is known that replacement of one quaternary moiety of the bis-quaternary ammonium structure by a primary amine group results in a considerable loss of potency (1), vecuronium possesses a powerful neuromuscular blocking action. Its principal value is expected to be as an adjunct to anaesthesia.

The neuromuscular blocking action of pancuronium has been well documented (2). The comparison of pharmacological activities of vecuronium to pancuronium was already done in some earlier studies (3–6), and the effectiveness of both drugs is almost the same; however, electrophysiological studies are lacking. In the present study, the inhibitory action of vecuronium on the frog end-plate potential was investigated using an electropharmacological technique.

In the last decade, electrophysiological experimentation has revealed the electrical event associated with the opening and closing of the individual receptor channels associated with activation by an agonist. This was achieved by statistical analysis of membrane potential or conductance fluctuations during continuous administration of agonist (7, 8) and more recently, by direct recording from a single receptor channel with a suction electrode on denervated skeletal muscle (9). Since the rate of decay of

Fig. 1. Chemical structure of vecuronium bromide: 1-[(2β, 3α, 5α, 16β, 17α)-3,17-bis(acetyloxy)-2-(1-piperidinyl)-androstane-16-yl]-1-methyl piperidinium bromide.
synaptically evoked currents is thought to reflect the average channel open time, measurement of the end-plate current (e.p.c.) is useful for elucidating agonist-receptor kinetics in the presence of an antagonist (8, 10–12). Recently, some competitive neuromuscular blockades and ganglion blocking agents were found to act in part as an open channel blocker at the vertebrate cholinergic nicotinic system (13–15) and to depress the glutamate response as well as the insect (16, 17) and crayfish neuromuscular junctions (18, 19). It is of great interest that cholinergic antagonists possess a property of channel blocking at the other transmitter systems. The crayfish neuromuscular junction provides an excellent model for studying the mechanism of action of drugs on synaptic transmission. In the present study, whether vecuronium acted as an open channel blocker at the crayfish neuromuscular junction or not was also examined by analyzing the time course of the e.j.c.

Materials and Methods

Drugs used: Acetylcholine chloride (ACh, Daiich Seiyaku), vecuronium bromide (Nippon Organon), pancuronium bromide (Sankyo) and monosodium L-glutamate (Wako Junyaku).

Frog experiment: The methods used were similar to those described in earlier papers (20). The nerve-sartorius preparation of the frog (Rana catesbeiana) was used, immersed in an isotonic solution containing (mM) NaCl, 113; KCl, 2; CaCl2, 1.8; NaHCO3, 1 and glucose, 2.8 (pH 6.9). In some experiments, the bathing medium was modified according to the nature of the experiment. A nerve bundle to the muscle was exposed and stimulated with a suction electrode to record the end-plate potential (e.p.p.). Potential changes of the muscle membrane were recorded with a 3 M KCI-filled microelectrode. The usual procedure was to locate a suitable spot with the internal electrode and record spontaneous potentials on moving film. The amplitude of the potentials were measured, and their distribution displayed in a histogram. In some experiments, the membrane potential of muscle fibers was clamped at the resting membrane potential with an intracellular microelectrode to record the end-plate current (e.p.c.). The clamp method was similar to that described by Takeuchi and Takeuchi (21). In order to measure the input resistance of the muscle fiber, two intracellular microelectrodes were inserted separately into the middle of a muscle fiber less than 50 µm apart, one for recording and the other for passing hyperpolarizing current. In an experiment, ACh was injected electrophoretically by applying a positive pulse to a micropipette containing 1 M ACh (>100 MΩ). Test samples were administered by bath application.

Crayfish experiment: The methods used were essentially similar to those reported previously (18).

The least squares procedures were performed with a desk-top computer (YHP 9845B). Experiments were made at a constant bath temperature of about 22°C. The results are presented as the mean values±S.E.M. for n experiments. Differences were analyzed using Student’s t-test to determine significant differences.

Results

Vecuronium depresses the postsynaptic response of the frog end-plate without affecting the presynaptic events: When the muscle fiber of the frog was treated with vecuronium bromide (2×10⁻⁶ g/ml), the resting membrane potential (−89.5±1.3 mV, n=6) was not affected by the drug. Vecuronium at a concentration of 2×10⁻⁶ g/ml did not produce a detectable change in amplitude of the electrotonic potential evoked by constant and hyperpolarizing current pulses (200 msec, 7 nA), suggesting that the drug did not affect the membrane resistance. After the saline had been replaced by the one containing vecuronium (2×10⁻⁶ g/ml), the amplitude of the spontaneous miniature end-plate potential (m.e.p.p.) was markedly reduced, and the spontaneous m.e.p.p. completely disappeared at higher concentrations of vecuronium, but recovery was complete and very prompt after washing the preparation. Figure 2 shows records of spontaneous m.e.p.p. in the absence and presence of vecuronium (10⁻⁶ g/ml). Figure 3 represents histograms of the amplitude distri-
bution of the spontaneous m.e.p.p. in different concentrations of vecuronium. The values of the mean amplitude of spontaneous m.e.p.ps were lowered as the concentration of vecuronium increased. On the other hand, the frequency of spontaneous m.e.p.ps was not affected by the drug, even when the concentration of vecuronium was increased up to $2 \times 10^{-6}$ mg/ml (Table 1).

When the nerve-muscle preparation was immersed in an isotonic solution containing of CaCl$_2$ and MgCl$_2$, adjusted so as to reduce the amplitude of e.p.ps to any desired level, vecuronium depressed the amplitude of the e.p.p. to about a half of the control at the concentration of $10^{-6}$ g/ml (Fig. 4). When various concentrations of vecuronium were added to the bathing medium, the amplitude of the e.p.p. was reduced in a dose-dependent

![Fig. 2. Effect of vecuronium bromide on the size of the m.e.p.p. The resting membrane potential was -89 mV. A: in normal Ringer, B: after addition of $10^{-6}$ g/ml vecuronium bromide. The size of the m.e.p.p. was reduced by vecuronium bromide without affecting its frequency.](image)

| Concentration (g/ml) | Number | Ratio (test / control) | Frequency |
|----------------------|--------|------------------------|-----------|
| $2 \times 10^{-7}$   | 1      | 0.91                   | 0.98      |
| $5 \times 10^{-7}$   | 3      | 0.81 ±0.08             | 1.06 ±0.12|
| $1 \times 10^{-6}$   | 6      | 0.63 ±0.05             | 0.94 ±0.04|
| $2 \times 10^{-6}$   | 1      | 0.37                   | 0.97      |

The amplitudes and frequencies of spontaneous m.e.p.ps in the presence of vecuronium were expressed as a ratio to the control. The mean resting membrane potential was $-89.5$ mV ±1.3 mV, $n=11$, and the mean value of amplitudes was $0.51±0.05$ mV ($n=11$) in the control. The frequency varied between 0.53/sec and 2.99/sec (mean $1.23±0.28$/sec).
manner. The degree of inhibition of nerve evoked e.p.ps was similar to that of spontaneous m.e.p.ps, suggesting that vecuronium depresses the postsynaptic response without affecting the presynaptic events of the frog end-plate. At concentrations greater than 2 × 10^{-6} g/ml, the e.p.p. sometimes completely disappeared. To compare the effect of vecuronium on e.p.ps with ACh potentials, ACh was applied for a fixed pulse duration of 1 msec in the absence and presence of different concentrations of vecuronium. At a concentration of 10^{-6} g/ml, vecuronium depressed the amplitude of the ACh potential to about 60% of the control level, and it was reduced in a dose-dependent manner.

**Vecuronium may act in part as an open channel blocker at the frog endplate:** When the interpulse interval of the double ACh pulse was long enough, the ACh pulse gave equal responses. However, the amplitude of the second ACh potential became smaller as the interpulse interval decreased. The interpulse interval was critically adjusted to give equal response, and effects of vecuronium on the amplitude of the double ACh potential were examined. In the presence of vecuronium (10^{-6} g/ml), the first ACh potential was reduced by vecuronium to about a half of the control, and the second was more markedly decreased than the first. This effect became more prominent when successive ACh potentials were induced by a train of ACh pulses. As shown in Fig. 5A, during a train of ACh pulses, successive ACh potentials gradually decreased in size in the presence of vecuronium. Therefore, it seems unlikely that vecuronium binds exclusively to the receptor in its resting state as a simple competitive antagonist does. The above results suggest that vecuronium binds to other receptors [AR and AR* in scheme (1)] as well in addition to the receptor in its resting state, for example, as an open channel blocker at the frog end-plate or an accelerator of the desensitization of the ACh receptor.

Since the decay rate of the e.p.c. is thought to reflect the average channel open time, the effect of vecuronium on the decay rate of the e.p.c. was investigated at various concentrations. The decay rate of the e.p.c. was not always affected by vecuronium in spite of the fact that the e.p.c. amplitude was significantly
decreased, but in some muscles, a slight shortening of the e.p.c. decline was observed at the resting membrane potential (Fig. 5B). Since the channel block theory predicts that transformation from the active open channel to the blocked open channel is a function of the blocker concentration, it is necessary to obtain clear results at high concentrations of blockers. However, in such a condition, the e.p.c. disappeared completely, and it was quite impossible to determine the decay rate of the e.p.c.

**Vecuronium depresses both the glutamate response and the excitatory junctional potential at the crayfish neuromuscular junction:** Vecuronium affected neither the resting membrane potential nor the input membrane resistance of the crayfish muscle fiber. Successive excitatory junctional potentials (e.j.ps) were induced by trains of pulses at 100 Hz for 80 msec and were recorded intracellularly. Vecuronium depressed the peak amplitude of intracellular successive e.j.ps in a dose-dependent manner. The effective dose range was markedly higher (above 100 times) than in the case of ACh responses, but it was almost similar to that in other established glutamate inhibitors at the crayfish neuromuscular junction. At a concentration of 1 mM (6.4×10^-4 g/ml) of vecuronium, the amplitude of e.j.ps and glutamate potentials induced by iontophoretically applied glutamate was reduced to about 20% of the control level. The amplitude of successive e.j.ps was decreased about half at a concentration of 0.5 mM, while the depolarization of the same muscle fiber produced by bath-applied glutamate (0.1 mM) was reduced to about 85% of the control.

**Vecuronium increases the decay rate of extracellularly recorded e.j.ps at the crayfish neuromuscular junction:** When the muscle fiber was treated with vecuronium, an evident change in extracellular e.j.ps was observed. The amplitude of extracellular e.j.ps was markedly decreased, and the decay rate of the tails of them significantly increased (Fig. 6). The number of quanta released per impulse, which was estimated from the number of failures of extracellular e.j.ps, scarcely changed (in an experiment, it changed from 0.051 to 0.053 in the presence of 0.5 mM vecuronium). Properties of extracellular e.j.ps have been studied in detail (22). The time constant of decay of extracellular e.j.ps was determined from the semilogarithmic plots of their decay phases by the method of least-squares. Regressions were calculated between 80% and 20% of the peak amplitude, and the plots were seen to fall close to a straight line. Figure 7 shows histograms of the time constant of the decay phase of extracellular e.j.ps in the absence and presence of vecuronium (0.5 mM) at the resting membrane potential. The extreme shortening with large doses of vecuronium indicates a change in the effective lifetime of the ion channel which cannot be explained by competitive binding. These results suggest a possibility that vecuronium acts as an open channel blocker at the crayfish neuromuscular junction.

If the reduction in peak amplitude of extracellular e.j.ps caused by vecuronium results from the open channel block, then the rise time of extracellular e.j.ps (the time to peak) should be shortened by vecuronium. The rise time of extracellular e.j.ps was 0.31±0.01 msec (n=88) and 0.20±0.01 msec (n=65) in the absence and presence of vecuronium, respectively. The difference was statistically significant at P<0.005. From these results, it is suggested that the open channel block almost fully accounts for the entire action of vecuronium at the crayfish
Pancuronium also depressed the amplitude of extracellular e.j.ps of the crayfish opener muscle and markedly decreased the decay time constant of extracellular e.j.ps. Pancuronium seemed to be more powerful than vecuronium. At a concentration of 0.2 mM, pancuronium decreased the decay time constant from 0.60 ± 0.02 (n=56) msec to 0.41 ± 0.02 (n=57) msec, and the rise time was changed from 0.38 ± 0.01 (n=56) msec to 0.29 ± 0.02 (n=57) msec. These differences were statistically significant at P<0.005.

**Discussion**

The results obtained in the present study demonstrated that vecuronium depressed both the ACh response at the frog end-plate and the glutamate response at the crayfish neuromuscular junction. Since the frequency of spontaneous m.e.p.ps was not affected in the presence of high concentrations of vecuronium and the degree of inhibition of m.e.p.p. amplitudes by vecuronium was similar to those of nerve evoked e.p.ps and ACh potentials, it can be concluded that vecuronium acts on the postsynaptic membrane. Strong evidence for the blocking of ion channels by cationic drug molecules has been obtained in the presence of permanently charged quaternary derivatives of lignocaine (23), while the uncharged compound also strongly blocks ACh-activated ion channels (24). Therefore, the channel blocking action of vecuronium is not necessarily due to the cationic quaternary ammonium moiety.

The principal action of vecuronium is probably of the competitive receptor blocking type because the ACh potential and spontaneous m.e.p.ps completely disappeared when the drug concentration increased, and vecuronium also shows an additional channel blocking action at the frog end-plate, like d-tubocurarine and pancuronium (2, 25-27). The model that has most commonly been proposed to account for the properties of this type of channel blocking is the following:

\[
A + R \rightleftharpoons AR \rightleftharpoons AR^* \\
\downarrow \alpha \ \beta \\
XR \grey{XR^*}
\]

In this scheme, A is an agonist molecule, R the receptor in its resting closed state, R* the receptor in its active open state, and X is the blocking agent. \(\alpha\) and \(\beta\) are the forward and backward rate constant, respectively. AR is the agonist-receptor complex associated with a closed ionic channel, and AR* is the open conformation of this complex. XR* is thus the active but blocked state of the channel. It has been reported that the values of \(\alpha\) and \(\beta\) are 7.4 and 0.86 at the frog membrane potential of -70 mV, respectively, in the case of d-tubocurarine (27).

If the value of \(\alpha\) is relatively large while that of \(\beta\) is small, it is possible to explain the...
experimental observation that the response to bath-applied glutamate was not so much reduced as the e.j.ps. The marked reduction of the decay time constant of extracellular e.j.ps showed a large value of $\alpha$. If the value of $\beta$ is relatively large, the decay phase should become double phasic as that of chlorisondamine does (19). In the case of bath application, the responses can be predicted to be in near equilibrium conditions. Therefore, in the presence of vecuronium, the depolarization of the crayfish muscle fiber caused by bath-applied glutamate may be affected by the equilibrium constant of the reaction $AR^* \rightleftharpoons XAR^*$. On the other hand, the depolarization in the relatively rapid phenomenon such as m.e.p.ps is affected by the magnitude of the rate constant $\alpha$, rather than the equilibrium constant, if the value of $\alpha$ is significantly larger than that of $\beta$, because such a rapid phenomenon is not in the equilibrium condition.

It is of great interest that vecuronium is expected to possess an additional open channel blocking action at the frog end-plate like other competitive antagonists. Until recently, it has been believed that many neuromuscular blocking agents are exclusively competitive antagonists for ACh. However, from the best experimental knowledge, they have a property for channel blocking as well. The channel block theory predicts that transformation from the active open channel ($AR^*$) to the blocked open channel ($XAR^*$) is a function of the blocker concentration. In the crayfish neuromuscular preparation, the high concentration of the blocker is required to depress the synaptic response, so the channel blocking action of vecuronium may be expected to appear in the crayfish preparation rather than at the frog end-plate. Many pharmacological agents have been reported to possess an additional channel blocking action at the vertebrate end-plate (14); however, not all these agents have been examined to have channel blocking properties at other synapse systems such as the crayfish and locust neuromuscular junction, and it is desirable to examine whether most of these agents show the property of channel blocking at the neuromuscular junction. From our data so far, the cholinergic nicotinic blockade seemed to possess such a property (18, 19).

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