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Open channel and competitive block of nicotinic receptors by pancuronium and atracurium

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Abstract

Mouse myotubes were used to investigate effects of the nondepolarizing neuromuscular blocking drugs pancuronium and atracurium on embryonic-type nicotinic acetylcholine receptor channels. Experiments were performed using patch-clamp techniques in combination with devices for ultra-fast solution exchange at outside–out patches. Application of 0.1 mM acetylcholine resulted in a fast current transient. When the peak amplitude was achieved, the current decayed monoexponentially due to desensitization. After application of drugs (pancuronium or atracurium), two different mechanisms of block were observed: (1) open channel block of embryonic-type nicotinic acetylcholine receptor channels after coapplication of blocker and acetylcholine, characterized by decrease of the time constant of current decay; (2) competitive block of embryonic-type nicotinic acetylcholine receptor channels by pancuronium or atracurium after preincubation of outside–out patches with the respective blocker. Different affinities of pancuronium ($K_B \approx 0.01$ μM) and atracurium ($K_B \approx 1$ μM) to embryonic-type nicotinic acetylcholine receptor channels were observed.

Keywords: Nicotinic receptor channel, embryonic-type; Atracurium; Pancuronium; Open channel block; Competitive block

1. Introduction

Competitive block (Jenkinson, 1960; Beranek and Vyskocil, 1968) of nicotinic acetylcholine receptor channels is supposed to be the main mechanism of action of non-depolarizing blocking drugs, which are widely used as muscle relaxants in anesthesia. Neuromuscular blocking drugs compete with acetylcholine for the binding site at nicotinic acetylcholine receptor channels (Lingle and Steinbach, 1988). In addition, like many local anesthetics and related drugs, neuromuscular blocking drugs can bind to the open state of that channels, resulting in open channel block (Ascher et al., 1978; Manalis, 1977). Recently, open channel and competitive block of nicotinic acetylcholine receptor channel currents by (+)-tubocurarine were analyzed quantitatively (Bufler et al., 1996b) and reactions of neuromuscular blocking drugs with nicotinic acetylcholine receptor channels were included into a circular kinetic reaction scheme of activation, desensitization and resensitization of embryonic-type nicotinic acetylcholine receptor channels (Franke et al., 1993).

In clinical practice, (+)-tubocurarine has been replaced by newer neuromuscular blocking drugs such as atracurium and pancuronium. The aim of the present study was to analyze mechanisms of block of these compounds at the molecular level.

2. Materials and methods

Patch-clamp experiments were performed on cultured myotubes from newborn mice, prepared as described elsewhere (Franke et al., 1992). Cells were kept in culture dishes at 37°C, 5% CO_2 for 7 to 14 days. Under these conditions, embryonic-type nicotinic acetylcholine receptors is expressed exclusively as indicated by the typical slope conductance of about 40 pS (Franke et al., 1992).

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Patch pipettes were pulled from borosilicate glass tubes with a DMZ-Universal Puller (Zeitz Instruments, Augsburg, Germany). They had a series resistance between 5 and 10 MΩ when filled with intracellular solution containing (in mM): 140 KCl, 11 EGTA, 10 HEPES, 10 glucose, 2 $MgCl_2$. The osmolarity was adjusted to 340 mosM l^{-1} with mannitol. Myotubes were superfused with an extracellular solution containing (in mM): 162 NaCl, 5.3 KCl, 0.67 $NaHPO_4$, 0.22 KH_2PO_4, 15 HEPES, 5.6 glucose. pH of solutions was adjusted to 7.3. Data were recorded with an EPC9 patch-clamp amplifier (List Electronics, Darmstadt, Germany). Ensemble currents were filtered at 2 kHz. Currents were recorded in the voltage clamp with a holding potential of −40 mV. Application of agonists was performed using a piezo-driven device for ultra-fast solution exchange (Franke et al., 1987). Neuromuscular blocking drugs were coapplied via the acetylcholine-containing test solution to test for open channel block or delivered from the background solution of the fast application system to investigate competitive block. 0.1 mM acetylcholine was applied before and after application of neuromuscular blocking drugs to test for reversibility of block and to get reference values of current amplitudes and time constants of current decay, τ. Time for exchange of the test solution was <100 μs (Bufler et al., 1996a; Jahn et al., 1998), exchange of the background solution took around 100 ms (Bufler et al., 1996b).

Acetylcholine and pancuronium were obtained from Sigma Aldrich (Deisenhofen, Germany) and atracurium from Glaxo Wellcome (Hamburg, Germany). Drugs were dissolved in extracellular solution prior to the experiments. All experiments were performed at room temperature (22–24°C). At pH 3.3 and 20°C, atracurium was shown to have very small degradation during a period of 3 months (Russell and Meyer-Witting, 1990). Degradation of atracurium decreased to 1 h at body temperature in human serum (Stiller et al., 1985). Under the experimental conditions of the study with atracurium dissolved in extracellular solution at pH 7.3 and room temperature, no reduction of the efficacy of atracurium was observed during a set of experiments with on average 4-h duration.

3. Results

Pulsed application of acetylcholine to excised outside–out patches from mouse myotubes results in current transients reaching maximal amplitudes at concentrations ≥ 0.1 mM acetylcholine. When antagonists have binding sites at the open state of a receptor, this type of block is denoted as open channel block. By using fast application techniques, open channel block of nicotinic acetylcholine receptor channels was demonstrated convincingly for different drugs (Bufler et al., 1996a,b; Scheller et al., 1996; Dilger et al., 1997; Scheller et al., 1997; Hertle et al., 1997; Krampfl et al., 2000). In Fig. 1 (upper traces), currents activated by 0.1 mM acetylcholine were shown as a control. The peak current amplitudes in these experiments were −60 (Fig. 1A) and −94 pA (Fig. 1B), corresponding to simultaneous opening of at least 37 and 59 single nicotinic acetylcholine receptor channels, respectively. When the peak current amplitude was reached, current transients decreased in presence of acetylcholine due to desensitization of nicotinic acetylcholine receptor channels with time constants of 76 or 61 ms, respectively. Open channel block can be studied by application of the agonist and antagonist together. If low concentrations pancuronium or atracurium (10 μM) were added to the acetylcholine-containing test solution of the reservoir of the fast application system, τ decreased whereas the peak current amplitude was not affected (Fig. 1, middle traces). At higher concentrations of neuromuscular blocking drugs, τ and the peak current amplitude decreased (Fig. 1, lower traces). The peak current amplitude was −60 pA in control and −42 pA in presence of 100 μM pancuronium, i.e. a reduction of amplitude of ∼30% occurred when pancuronium was added to the test solution in this experiment (Fig. 1A, lower traces). Similar results were obtained when atracurium was added to the test solution. Under conditions of ultrafast solution exchange, the decrease of τ in presence of neuromuscular blocking drugs points to an open channel block mechanism. It is a measure for the binding rate constant to the open state of embryonic-type nicotinic acetylcholine receptor channels. There was no difference between pancuronium or atracurium with re-

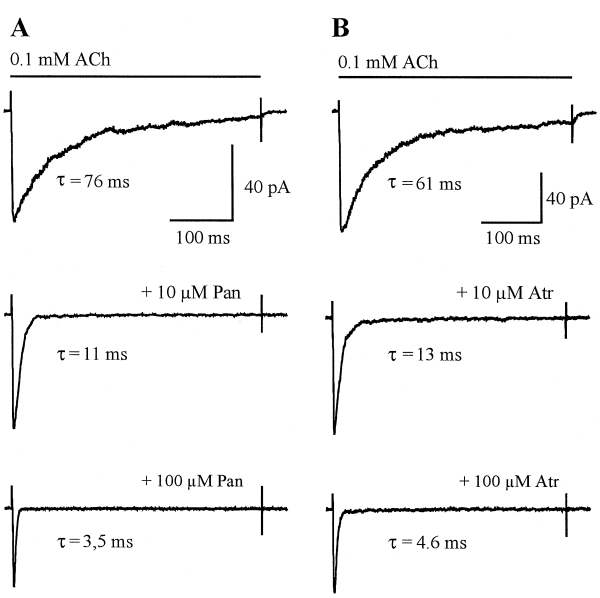

Fig. 1. Average currents of one outside–out patch, elicited by 400 ms pulses of 0.1 mM acetylcholine in combination with different concentrations of pancuronium (A) and atracurium (B) added to the acetylcholine-containing extracellular solution as indicated. Each trace is the average of 3–6 single current traces.

spect to the open channel block of embryonic-type nicotinic acetylcholine receptor channels. τ was 4.8 ms $\pm$ 2.5 S.D. ($n = 12$) and 4.9 ms $\pm$ 2.6 S.D. ($n = 11$) after application of 100 μM pancuronium or atracurium, respectively. These values were very close to that published for (+)-tubocurarine (Bufler et al., 1996b). The reduction of the peak current amplitude after application of high concentrations of acetylcholine + neuromuscular blocking drugs (lower traces of Fig. 1A and B) may be partly caused by interaction of neuromuscular blocking drugs with the unliganded state of the receptor (see Bufler et al., 1996a,b).

In order to study binding of neuromuscular blocking drugs to the non-liganded state of the receptor (competitive block), experiments were performed with preincubation of outside–out patches via the background flow of the fast application system containing the drug. The peak current amplitude was determined after pulsed application of 0.1 mM acetylcholine before, during and after preincubation of outside–out patches with different neuromuscular blocking drugs concentrations. Binding of pancuronium or atracurium to embryonic-type nicotinic acetylcholine receptor channels resulted in a marked, concentration-dependent reduction of the peak current amplitude, while activation and desensitization kinetics were not affected (Fig. 2A,C). The concentration of neuromuscular blocking drugs for effective competitive block of embryonic-type nicotinic acetylcholine receptor channel currents was markedly lower than the blocker concentration resulting in open channel block (compare Figs. 1 and 2B,D). No difference between pancuronium and atracurium was observed at open channel block. In contrast, competitive block of embryonic-type nicotinic acetylcholine receptor channels by pancuronium and atracurium revealed marked differences (Fig. 2). The peak current amplitude decreased by about 40% of control after application of 0.01 μM pancuronium. For the same block effect by atracurium, a 100-fold higher concentration must be applied (Fig. 2A,C, middle traces). As open channel block, competitive block by neuromuscular blocking drugs was fully reversible (Fig. 2A,C, lower traces). Concentration inhibition curves are shown in Fig. 2B and D. Complete block of embryonic-type nicotinic acetylcholine receptor channel currents was observed when outside–out patches were preincubated with 1 μM pancuronium or 100 μM atracurium, whereas a 50% block, corresponding to K_B, occurred at $\sim$0.01 μM pancuronium or $\sim$1 μM atracurium, respectively.

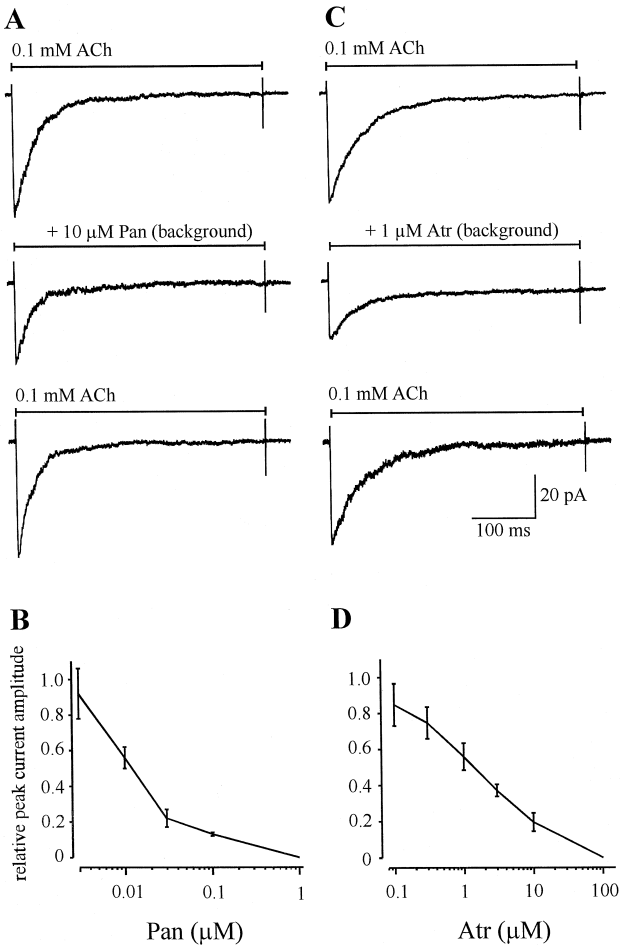

Fig. 2. Average currents from outside–out patches activated by 0.1 mM acetylcholine with different concentrations of pancuronium (A) and atracurium (C) added to the background solution (preincubation) as indicated. Lower traces show control pulses with 0.1 mM acetylcholine after wash-out of pancuronium or atracurium, respectively. Each trace was the average of 3–8 single current traces. The diagrams show concentration inhibition curves of the current activated by 0.1 mM acetylcholine in combination with increasing concentration of pancuronium (B) or atracurium (D). Each point is the average of six independent experiments.

As shown for (+)-tubocurarine (Bufler et al., 1996b), binding and unbinding of neuromuscular blocking drugs to/from unliganded nicotinic acetylcholine receptor channels (competitive block) proved to be slow compared to the kinetics of activation, desensitization and open channel block of nicotinic acetylcholine receptor channel currents. To investigate the time course of recovery from block, 20 ms pulses of 0.1 mM acetylcholine were applied repetitively after switching the background solution from a solution containing neuromuscular blocking drugs (1 μM pancuronium or 100 μM atracurium) to a background solution without any drugs added. The current returned to the original amplitude after an wash-out interval of 10–12 s (Fig. 3). The approximate time constants for unbinding of pancuronium and atracurium from the receptor were 7.6 and 6.7 s, respectively. These values, corresponding to unblocking rates of 0.13 (pancuronium) and 0.15 s^{-1} (atracurium), were in the same range as the unbinding rates of (+)-tubocurarine (Bufler et al., 1996b). With $K_B = 0.01$ μM (pancuronium) and $K_B = 1$ μM (atracurium) and the rate constants of unbinding as determined experimentally, the binding rate constants to the competitive blocking site of the receptor were calculated as 0.13×10^8 M^{-1} s^{-1}

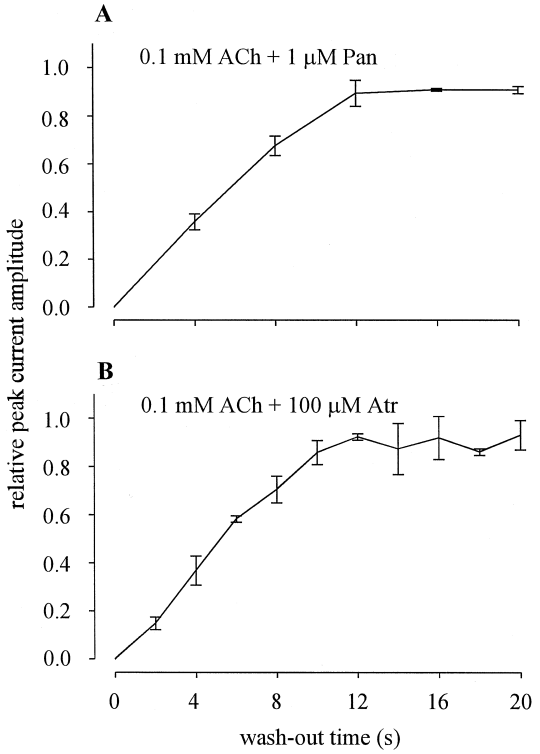

Fig. 3. Time course of recovery from block after preincubation of outside–out patches with 0.1 mM pancuronium (A, pulse frequency 0.25 Hz) or atracurium (B, pulse frequency 0.5 Hz). 20 ms pulses of 0.1 mM acetylcholine were applied. Each point is the average of six independent experiments.

(pancuronium) or 0.15×10^6 M^{-1} s^{-1} (atracurium), respectively, according to the equation $b_1 = b_{-1}/K_B$.

4. Discussion

On the molecular level, neuromuscular blocking drugs exhibit three different modes of action at nicotinic acetylcholine receptor channels: (a) competitive block (Jenkinson, 1960; Bufler et al., 1996b), (b) open channel block (Adams, 1977; Manalis, 1977; Ascher et al., 1978; Colquhoun et al., 1979; Bufler et al., 1996b) and (c) direct activation of nicotinic acetylcholine receptor channels (Trautmann, 1982; Steinbach and Chen, 1995). There are profound differences in dosage and plasma levels between different neuromuscular blocking drugs (Rowland, 1998). Open channel block of nicotinic acetylcholine receptor channel currents by neuromuscular blocking drugs is not expected to play a major role in muscle relaxation, because the time course of excitatory postsynaptic potentials with decay time constants in the range of some milliseconds due to disintegration of acetylcholine is much faster than τ in presence of neuromuscular blocking drugs in concentrations < 100 μM. Therefore, at low concentrations of neuromuscular blocking drugs, no reduction of the postsynaptic current as a result of open channel block is expected. In contrast, a marked decrease of the peak current amplitude elicited by 0.1 mM acetylcholine was observed after application of low concentrations of pancuronium or atracurium via the background solution of the fast application system (Fig. 2). It is therefore suggested that competitive block of nicotinic receptors, i.e. binding to the unliganded state of the receptor, is most important in neuromuscular block by NDB.

Recently, the interaction between acetylcholine, (+)-tubocurarine and nicotinic acetylcholine receptor channels was investigated. An extended circular kinetic scheme for activation, desensitization and block of the nicotinic acetylcholine receptor channels was proposed and the rate constants in this scheme were determined quantitatively (Bufler et al., 1996a,b). In the present study, interactions between pancuronium or atracurium, acetylcholine and embryonic-type nicotinic acetylcholine receptor channels were measured. No significant differences were observed in open channel block of nicotinic receptors between pancuronium, atracurium or (+)-tubocurarine (see Fig. 1; Bufler et al., 1996b). The most important finding of the study was that the affinity of neuromuscular blocking drugs to nicotinic receptors differed by nearly two orders of magnitude. This may reflect different binding rate constants of pancuronium and atracurium to the unliganded receptor state (Fig. 2B,D), whereas unbinding rate constants investigated by wash-out experiments did not show significant difference. The data of our study were obtained from native embryonic-type nicotinic acetylcholine receptor channels. Recently, similar block mechanisms (open channel block and competitive block) were also observed in recombinant adult-type nicotinic channels (Krampfl et al., 2000). Therefore, it is assumed that embryonic- and adult-type nicotinic acetylcholine receptor channels share the principles of open channel and competitive block.

Under clinical conditions, in the synaptic cleft there may be an equilibrium between the unliganded and blocked state of the receptor in presence of neuromuscular blocking drugs. The dosage necessary to obtain sufficient muscle relaxation is considerably lower for pancuronium than for atracurium (Rowland, 1998). Among other reasons, this is supposedly a consequence of the higher binding rate constant, and thus, lower K_B of pancuronium.

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