Anesthesiology
1998; 88:1003-13

Effects of Neuromuscular Blocking Agents on Excitatory Transmission and γ-Aminobutyric Acid$_A$-mediated Inhibition in the Rat Hippocampal Slice

Florence C. Chiodini, Edömer Tassonyi, M.D., Ph.D.,† Thomas Fuchs-Buder, M.D.,‡ Marc Fathi, Ph.D.,§ Daniel Bertrand, Ph.D.,‖ Dominique Muller, M.D., Ph.D.#*

Background: **Although neuromuscular blocking agents do not cross the blood–brain barrier, they may penetrate the central nervous system under particular circumstances and eventually cause neurotoxic consequences.**

Methods: **The effects of neuromuscular blocking agents on excitatory and inhibitory transmission in area CA1 of rat hippocampal slices were investigated using extracellular and intracellular recording techniques.**

Results: **Application of atracurium in the perfusion medium resulted in a dose-dependent enhancement of excitatory synaptic responses averaging 48.7 ± 4.3% at a concentration of 10 nM. This effect was correlated with an increase in the size of the presynaptic fiber volley. Laudanosine, but not pancuronium bromide or vecuronium bromide, produced similar changes. In addition, atracurium and laudanosine blocked inhibitory transmission and reduced intracellularly recorded γ-aminobutyric acid$_A$ receptor-mediated potentials. These effects were observed only at concentrations >1 μM and were not reproduced by pancuronium bromide and vecuronium bromide.**

Conclusions: **Atracurium and its metabolite, laudanosine, contrary to pancuronium bromide and vecuronium bromide, produce two distinct effects on hippocampal slices. They enhance excitatory transmission and neuronal excitability and they block inhibitory γ-aminobutyric acid$_A$-mediated synaptic responses. (Key words: Atracurium; CA1; excitatory postsynaptic field potentials; inhibitory postsynaptic potentials; laudanosine.)**

QUATERNARY ammonium compounds such as neuromuscular blocking (NMB) agents are considered not to cross the blood-brain barrier.[1] However, under certain circumstances, muscle relaxants may be found in the cerebrospinal fluid (CSF) of patients. Matteo *et al.*[2] found d-tubocurarine in lumbar CSF after intravenous administration in patients undergoing craniotomy for cerebral aneurysms, arteriovenous malformations, and pituitary tumors. Eddleston *et al.*[3] detected atracurium (up to 1,460 ng/ml; 1.2 μM) in the CSF of 2 of 10 patients with subarachnoid hemorrhage. Laudanosine, the major metabolite of atracurium, was also reproducibly recovered in the CSF of these patients in concentrations that ranged from 2-570 ng/ml (0.005-1.6 μM).[3,4] Therefore, it was suggested that most muscle relaxants probably cross the blood-brain barrier when administered in large doses in critically ill patients.[5]

The potential problems that may result from a possible crossing of the blood-brain barrier by NMB agents are highlighted by several studies suggesting that these compounds are pharmacologically active in the central nervous system (CNS). Autonomic dysfunction or weakness, prolonged neuromuscular blockade, neuronal death, and seizures have all been reported.[6-10] Administration of NMB agents such as vecuronium bromide, pancuronium bromide, or atracurium to rats has been reported to produce a dose-dependent excitation of the CNS progressing to seizures.[10] *In vitro* experiments done using hippocampal slices or neocortical slices have shown that NMB agents can trigger epileptiform activity.[11-12] The epileptiform activity reported in brain slices is compatible with both interactions with excitatory and inhibitory transmission. Alternatively, recent studies have also proposed that the dose-dependent CNS excitement produced by vecuronium bromide and

* Doctoral Student, Neuropharmacology.

† Staff Anesthesiologist, Division of Anesthesiology.

‡ Chief Resident, Division of Anesthesiology.

§ Staff Chemist, Clinical Chemistry of Central Laboratory.

‖ Professor of Physiology.

Professor of Neuropharmacology.

Received from the Department of Anesthesiology, Pharmacology and Intensive Care Unit and the Department of Physiology, Centre Médical Universitaire, Geneva, Switzerland. Submitted for publication June 10, 1997. Accepted for publication November 21, 1997. Supported by Swiss National Research Foundation, grants 3200.043626.95 and 3100.040815.94 and de Reuter Foundation, Geneva, Switzerland. Presented in part at the annual meeting of the European Society of Anaesthesiologists, London, U.K., June 1996.

Address reprint requests to Ms. Chiodini: Neuropharmacology, Centre Médical Universitaire, 1 rue Michel Servet, 1211 Geneva 4. Address electronic mail to: Florence.Chiodini@medecine.unige.ch

pancuronium bromide could be due to an increase of intracellular cytosolic calcium.[13,14] Together these results clearly indicate that exposure of brain tissue to NMB agents may be associated with neurotoxic effects.

The precise pharmacologic action of NMB agents in the CNS is poorly understood, and the level (pre- or postsynaptic) and the nature (which receptor type, which mechanisms) of these effects are still essentially unknown. One possibility is that NMB agents interact with the recently discovered neuronal nicotinic acetylcholine receptors. Molecular studies have provided evidence for the existence of different subtypes of receptors, and recent results suggest that these receptors are involved in the modulation of neurotransmission. Given the possible clinically relevant effects of these compounds, it appeared important to analyze further the mechanisms of action of NMB agents in the brain. The present study investigated the effects of two classes of NMB agents, the benzylisoquinolonium derivative atracurium and its metabolite laudanosine, and the steroid compounds pancuronium bromide and vecuronium bromide.

Materials and Methods

Slice Preparation

Hippocampal slices (400-μm thick) were prepared from 3- to 4-week-old Sprague-Dawley rats using a protocol approved by the Geneva Veterinarian Office (authorization 31.1.1024/1190/0). The animals were anesthetized with enflurane and decapitated with a guillotine. The brain was quickly removed and cooled in ice-cold saline, and slices were prepared using a McIlwain chopper. The slices were maintained in an interface chamber with a humid atmosphere composed of 95% oxygen and 5% carbon dioxide and continuously perfused (1 ml/min) with an artificial medium containing 124 mM NaCl, 1.6 mM KCl, 2.5 mM $CaCl_2$, 1.5 mM $MgCl_2$, 24 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 10 mM glucose, and 2 mM ascorbic acid, at *p*H 7.4. The temperature was maintained at 33°C. Slices were allowed to recover for at least 2 h before being tested.

Electrophysiologic Recording and Stimulation

Extracellular and intracellular recordings were made in area CA1 with glass electrodes pulled from capillaries (GC120F-10; Clark Electromedical Instruments, UK) and filled with perfusion medium or 3 M potassium acetate (Sigma Chemical Co., St. Louis, MO) for extracellular (tip resistance, 3–10 MΩ) and intracellular (tip resistance, 25–80 MΩ) recording electrodes, respectively. Test stimuli were delivered *via* bipolar stimulating electrodes (two twisted strands of nichrome wires, 50 μm in diameter) positioned in the stratum radiatum of the CA1 region to activate Schaffer collaterals. Excitatory postsynaptic field potentials (EPSPs) were recorded in stratum radiatum of area CA1 and elicited by orthodromic paired activation of Schaffer collaterals using a 50-ms interpulse interval to generate paired-pulse facilitation (stimulation pulses: 1–10 μA, 200 μs in duration, delivered every 30 s; fig. 1). To assess inhibitory transmission, responses (EPSP and population spike [PS]) were recorded in the CA1 pyramidal cell layer and elicited by sequential stimulation at a 30-ms interval of two independent groups of Schaffer collaterals using two different stimulation electrodes. Independence of the two inputs was assessed by verifying the absence of heterosynaptic facilitation. Inhibition was analyzed by recording the reduction of the PS elicited in the pyramidal cell layer on the test input by previous activation 30 ms earlier of a conditioning pathway that triggered GABAergic inhibition (stimulation pulses: 1–10 μA, 200 μs in duration, delivered every 30 s; fig. 1). The degree of blockade of this inhibitory mechanism by NMB agents was quantified in table 1 by measuring the changes in PS amplitude that occurred before and during drug application and by calculating the ratio of these two measures. Isolated $GABA_A$-mediated inhibitory postsynaptic potentials (IPSPs) were recorded extracellularly in the presence of the excitatory amino-acid receptor antagonists CNQX and D-AP5, as described by Lambert *et al.*[15] For intracellular recordings, IPSPs were elicited by orthodromic activation of Schaffer collaterals and recorded in the CA1 pyramidal cell layer. Stimuli were single pulses (200 μs duration, every 15 s; fig. 1).

Drug Application

The following drugs were used: atracurium (Wellcome, UK), laudanosine (Sigma), pancuronium bromide (Sigma), vecuronium bromide, and 3-desacetyl-[OH]-vecuronium (Organon Teknika, The Netherlands). They were added to the perfusion medium at concentrations between 1 nM and 300 μM for 15–30 min depending on the experiments. 6-cyano-7-nitroquinoxaline-2.3,-dione (CNQX) and D(−)-2-amino-5-phosphonopentanoic acid (D-AP5) were obtained from Tocris (UK).

Data Analysis

Recorded signals were amplified (50–500×), filtered (DC-3.0 kHz, bandpass) and digitized on-line for com-

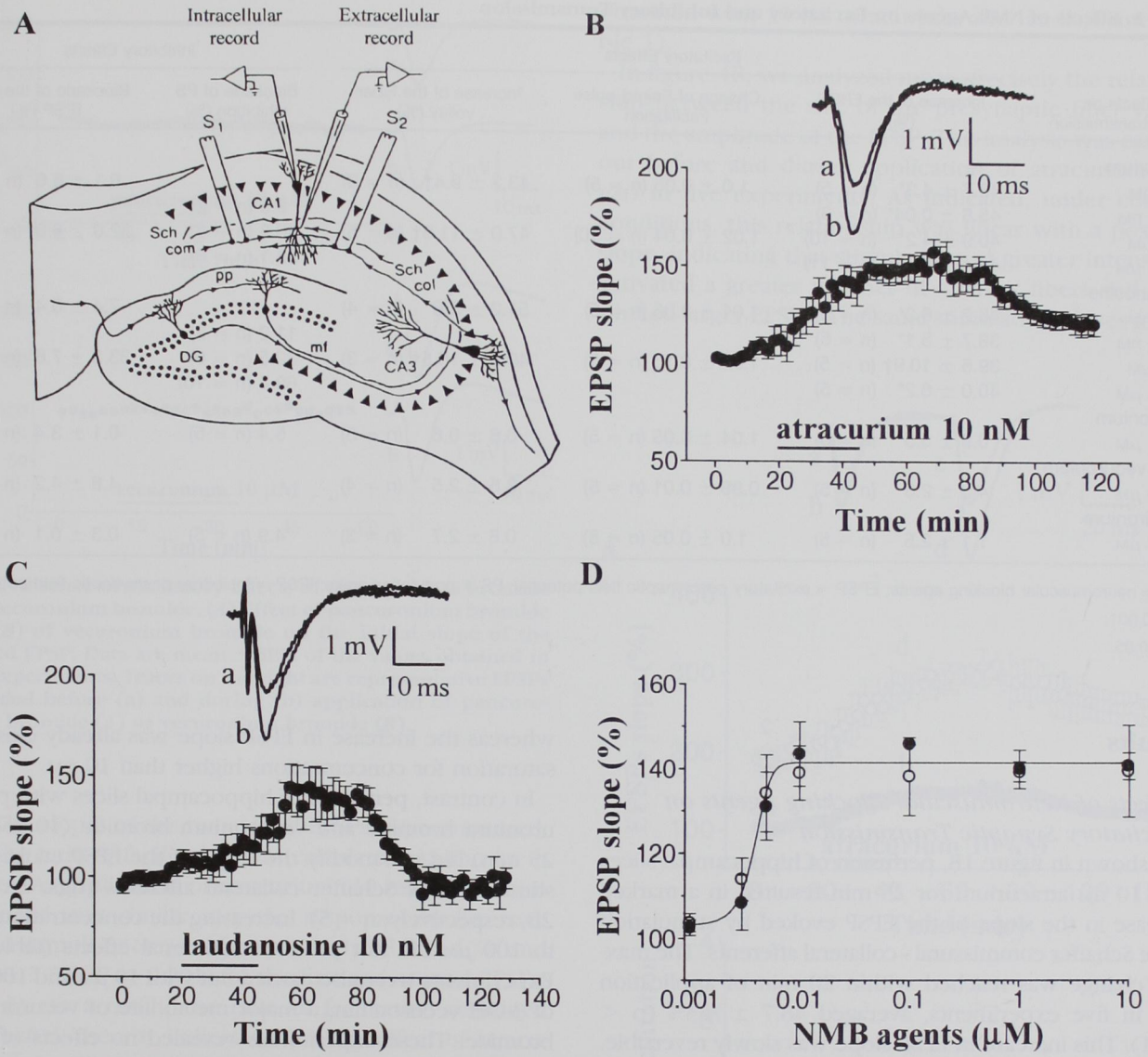

Fig. 1. Enhancement by atracurium and laudanosine of excitatory transmission in the hippocampus. (*A*) Schematic diagram illustrating the position of the stimulating (S1 and S2) and recording electrodes in a transverse hippocampal slice. EPSPs were recorded in CA1 stratum radiatum by orthodromic paired activation of Schaffer collaterals pathways at 50-ms intervals. For analysis of inhibition, the recording electrode was placed in the stratum pyramidale either extra- or intracellularly. (*B*) Initial slope of EPSPs measured before, during, and after perfusion of atracurium (*black bar*). Data are expressed as percent of baseline pre-drug values and represented as mean ± SEM (n = 5). Inset responses are representative traces recorded in one experiment before (a) and during (b) atracurium application. (*C*) Effect of laudanosine on the initial slope of the EPSP. Data are mean ± SEM of five experiments. Traces are representative responses recorded in one experiment before (a) and during (b) laudanosine application. (*D*) Dose–response curves for the effects of atracurium (*black circles*) and laudanosine (*open circles*) on the EPSP slope.

puter storage and data analysis. Parameters of recorded responses (slope, amplitude) were measured and plotted on-line using a computer program. PS amplitudes were measured from peak negativity to peak positivity. Preparations were usually exposed to only one concentration of a given NMB agent, to avoid possible residual drug effects. Data in all experiments were expressed as percent of baseline pre-drug values, they were averaged across experiments and calculated as means ± SEM; n refers to the number of slices tested per condition; only one slice per animal was included in the statistical analyses. Statistical analyses were carried out using the Student's *t* test. The threshold for significance was a probability value < 0.05.

Table 1. Effects of NMB Agents on Excitatory and Inhibitory Transmission

Effects on Neurotransmission	Excitatory Effects			Inhibitory Effects	
	Increase of the EPSP (%)	Change of Paired-pulse Facilitation	Increase of the Fiber-volley (%)	Blockade of PS Inhibition (%)	Blockade of the Field IPSP (%)
Atracurium					
10 nM	48.7 ± 4.3* (n = 5)	1.0 ± 0.05 (n = 5)	43.3 ± 9.4† (n = 3)		0.1 ± 6.6 (n = 5)
100 nM	45.8 ± 0.04* (n = 3)			2.5 (n = 2)	
10 μM	40.9 ± 4.2* (n = 10)	1.02 ± 0.04 (n = 10)	47.0 ± 11.8† (n = 5)	36.6 (n = 3)	32.0 ± 8.0* (n = 4)
100 μM	42.4 ± 3.4* (n = 11)			53.1 (n = 6)	
Laudanosine					
10 nM	46.2 ± 6.3* (n = 5)	1.01 ± 0.06 (n = 5)	51.2 ± 5.6* (n = 4)		7.4 ± 0.4 (n = 5)
100 nM	38.7 ± 5.1* (n = 5)			11.2 (n = 2)	
10 μM	39.6 ± 10.9† (n = 5)	1.02 ± 0.16 (n = 5)	49.5 ± 16.5† (n = 3)	32.5 (n = 6)	33.3 ± 7.6* (n = 4)
100 μM	40.0 ± 6.2* (n = 5)			69.4 (n = 10)	
Vecuronium					
100 μM	0.9 ± 3.5 (n = 5)	1.04 ± 0.05 (n = 5)	0.8 ± 0.6 (n = 5)	5.4 (n = 5)	0.1 ± 3.4 (n = 3)
3-OH vecuronium					
100 μM	4.3 ± 2.3 (n = 5)	0.98 ± 0.01 (n = 5)	3.6 ± 2.5 (n = 4)		4.8 ± 4.2 (n = 3)
Pancuronium					
100 μM	1.1 ± 2.5 (n = 5)	1.0 ± 0.05 (n = 5)	0.6 ± 2.7 (n = 3)	4.9 (n = 5)	0.3 ± 0.1 (n = 3)

NMB = neuromuscular blocking agents; EPSP = excitatory postsynaptic field potential; PS = population spike; IPSP = inhibitory postsynaptic field potential.

* $P < 0.001$.

† $P < 0.05$.

Results

Effects of Neuromuscular Blocking Agents on Excitatory Synaptic Transmission

As shown in figure 1B, perfusion of hippocampal slices with 10 nM atracurium for 25 min resulted in a marked increase in the slope of the EPSP evoked by stimulation of the Schaffer commissural-collateral afferents. The maximal change was reached within 20 min of application and, in five experiments, averaged 48.7 ± 4.3% ($p < 0.001$). This increase in EPSP slope was slowly reversible. As illustrated in fig. 1B, responses were still 21% larger than pre-drug values 1 h after washout. A similar change in EPSP slope was also obtained after application of 10 nM laudanosine for 25 min (averaged increase: 46.2 ± 6.3%; n = 5 slices; $P < 0.001$; fig. 1C). The effects of laudanosine were slightly slower to occur than those of atracurium and were fully reversible within 1-2 h of washout. Similar results were obtained with higher concentrations of either atracurium or laudanosine (table 1).

The effects of atracurium and laudanosine were clearly dose-dependent. Figure 1D shows the dose-response curves obtained with atracurium and laudanosine when measuring the increase in EPSP slope. Each point represents a mean ± SEM of 3-11 experiments. Little or no effect was observed with concentrations lower than 3 nM, whereas the increase in EPSP slope was already close to saturation for concentrations higher than 10 nM.

In contrast, perfusion of hippocampal slices with pancuronium bromide and vecuronium bromide (10 μM for 25 min) did not modify the slope of the EPSP evoked by stimulation of Schaffer collateral afferents (figs. 2A and 2B, respectively; n = 5). Increasing the concentrations up to 100 μM did not produce additional effects (table 1). Experiments were also carried out with 10 μM and 100 μM of 3-OH vecuronium, a major metabolite of vecuronium bromide. These experiments revealed no effects of this metabolite on the EPSP slope (table 1; n = 5).

Increase in Neuronal Excitability Produced by Atracurium and Laudanosine

The enhancement of the EPSP slope observed after perfusion with atracurium or laudanosine could be the result of either a selective effect of the drugs on glutamatergic transmission or a nonspecific increase in general excitability of the tissue. To investigate these possibilities, we first analyzed whether the changes in EPSP slope were associated with changes in paired-pulse facilitation, a property shown in many systems to be affected by modifications of release mechanisms.[16,17]

For these experiments, paired stimuli were delivered to Schaffer collaterals using an interpulse interval of 50

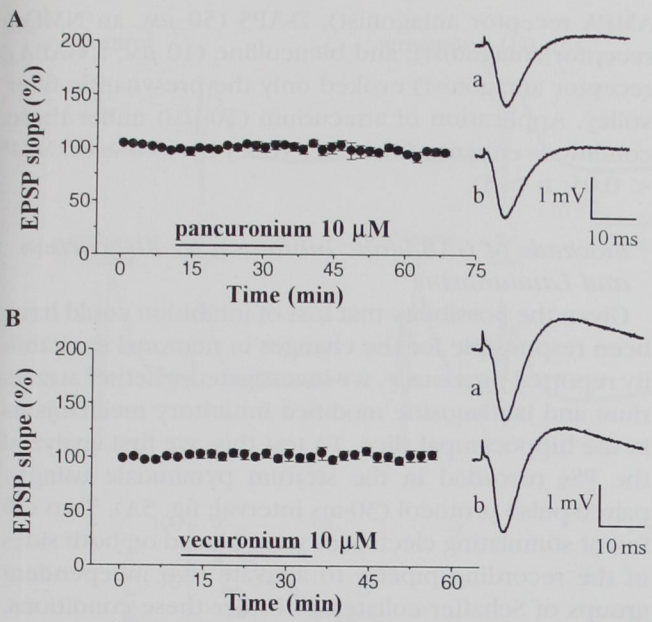

Fig. 2. Absence of excitatory effects of pancuronium bromide and vecuronium bromide. (*A*) Effect of pancuronium bromide and (*B*) of vecuronium bromide on the initial slope of the evoked EPSP. Data are mean ± SEM of the values obtained in five experiments. Traces on the right are representative EPSPs recorded before (a) and during (b) application of pancuronium bromide (*A*) or vecuronium bromide (*B*).

ms. The slopes of the two-paired EPSPs were measured, and the ratio of facilitation was calculated. As shown in figure 3A, application of atracurium (10 nM for 25 min) enhanced in a similar way the responses to the two-paired stimuli (increase of respectively 48.7 ± 4.3% and 48.8 ± 7.7%; $P < 0.001$, for the first and second EPSPs; n = 5). As a result, the ratio of facilitation remained unaffected, thereby indicating that the effect of atracurium was unlikely to involve modifications of transmitter release (fig. 3B). Similar results were also obtained with 10 μM atracurium and laudanosine (table 1).

As a second test, we then measured the size of the presynaptic fiber volley, a small deflection of the field response that precedes the postsynaptic potential and reflects presynaptic currents. The fiber volley correlates with the number of presynaptic afferents activated by the stimulation pulse (fig. 4). As shown in figure 4A, perfusion of atracurium (10 μM for 25 min) resulted in a marked increase of the EPSP amplitude but also of the amplitude of the presynaptic fiber volley. The changes in amplitude of the presynaptic fiber volley measured in a group of five experiments averaged 47.0 ± 11.8%, $P < 0.05$. Similar results were obtained with 10 nM atracurium and laudanosine (table 1).

In figure 4B, we analyzed more precisely the relationship between the size of the presynaptic fiber volley and the amplitude of the EPSP. This analysis was carried out before and during application of atracurium (10 μM) in five experiments. As indicated, under control conditions, this relationship was linear with a positive slope, indicating that stimulations of greater intensities activated a greater number of afferent fibers and thus evoked larger EPSPs. The same stimulation pulses in the

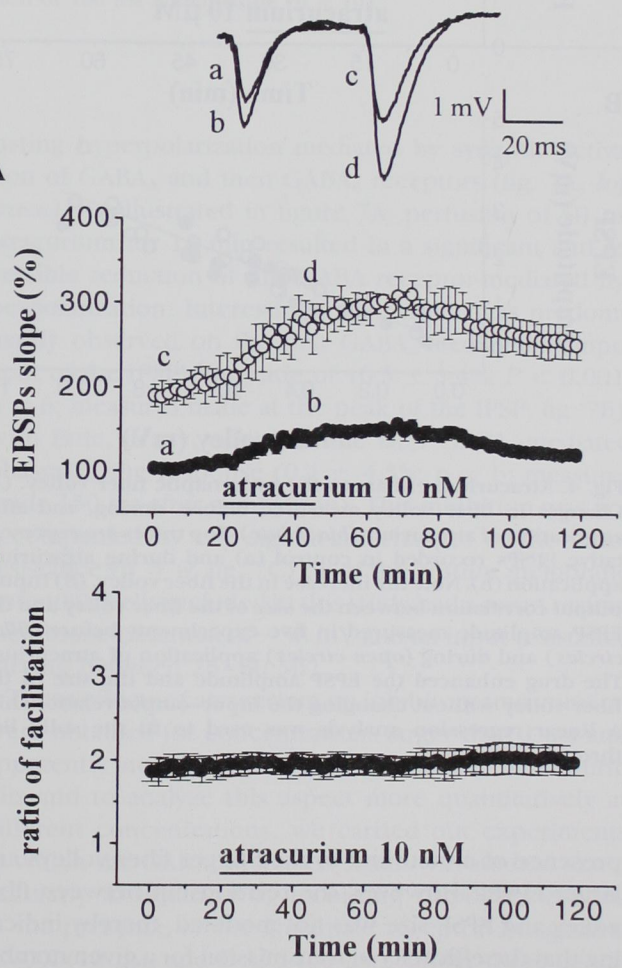

Fig. 3. Paired-pulse facilitation is not affected by atracurium. (*A*) Graph showing the changes in slope of the first (a,b) and second EPSPs (c,d) evoked by orthodromic paired activation of a group of Schaffer collaterals at 50-ms intervals. Data are expressed as percent of baseline values for the first EPSP. They are mean ± SEM of five experiments. Inset traces are representative field potentials recorded before (a,c) and during (b,d) atracurium application. (*B*) Ratio of facilitation (calculated as the ratio of slopes of the second over the first EPSP).

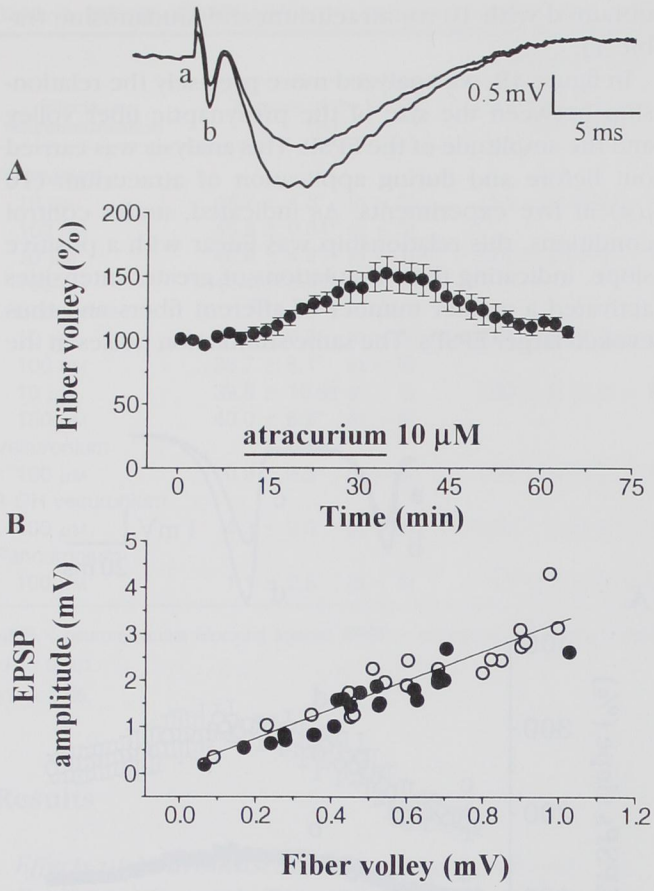

Fig. 4. Atracurium enhances the presynaptic fiber volley. (*A*) Changes in fiber volley measured before, during, and after application of atracurium (*black bar*). Top traces are representative EPSPs recorded in control (a) and during atracurium application (b). Note the increase in the fiber volley. (*B*) Input–output correlation between the size of the fiber volley and the EPSP amplitude measured in five experiments before (*filled circles*) and during (*open circles*) application of atracurium. The drug enhanced the EPSP amplitude and the size of the fiber volley without changing the input–output relationship. A linear regression analysis was used to fit the solid line through data points.

presence of atracurium evoked larger fiber volleys and larger EPSPs. However, the relationship between fiber volley and EPSP size was not modified, thereby indicating that the efficacity of transmission for a given number of activated synapses remained constant.

To further strengthen this conclusion and to eliminate the possibility of polysynaptic effects, experiments were also carried out by measuring the size of the presynaptic fiber volley under conditions of complete blockade of synaptic transmission. Activation of Schaffer collaterals in the presence of CNQX (10 μM, an AMPA receptor antagonist), D-AP5 (50 μM, an NMDA receptor antagonist), and bicuculline (10 μM, a $GABA_A$ receptor antagonist) evoked only the presynaptic fiber volley. Application of atracurium (10 μM) under these conditions enhanced the fiber volley by 45.0 ± 8.7% ($P < 0.05$; n = 3).

Blockade of GABAergic Inhibition by Atracurium and Laudanosine

Given the possibility that loss of inhibition could have been responsible for the changes in neuronal excitability reported previously, we investigated whether atracurium and laudanosine modified inhibitory mechanisms in the hippocampal slice. To test this, we first analyzed the PSs recorded in the stratum pyramidale using a paired-pulse protocol (30-ms interval; fig. 5A). Two different stimulating electrodes were placed on both sides of the recording pipette to activate two independent groups of Schaffer collaterals. Under these conditions, the PS generated by the test stimulation was reduced by the inhibitory GABAergic mechanisms elicited 30 ms earlier by the conditioning stimulus (see fig. 5A; control). After application of atracurium (100 μM; n = 6; fig. 5A), this inhibitory effect of the conditioning stimulation was strongly reduced. This blockade of inhibitory mechanisms is shown more quantitatively in the graph of figure 5B, which illustrates the changes in PS amplitude on the test input produced in six experiments by conditioning stimulations of increasing intensity. Under control conditions (*filled circles*), the presence of functional inhibitory mechanisms is reflected by a progressive reduction in the amplitude of the PS on the test input. After application of atracurium (100 μM for 30 min; n = 6; *open circles;* fig. 5B), this inhibition was markedly reduced. Figure 5C illustrates the same analysis carried out before (*filled circles*) and after application of laudanosine (100 μM, n = 10, *open circles*). As shown, very similar results were obtained, indicating that laudanosine also consistently blocked inhibitory mechanisms in the hippocampal slice.

Results from similar experiments carried out in the presence of pancuronium bromide and vecuronium bromide are shown in figure 6. As illustrated, application of pancuronium bromide and vecuronium bromide at concentrations of 100 μM for 30 min (figs. 6B and 6C, respectively; n = 5) did not alter the inhibitory effect of the conditioning stimulation on the PS elicited on the second test input.

To confirm the results obtained with atracurium and laudanosine, and to further characterize their effects on

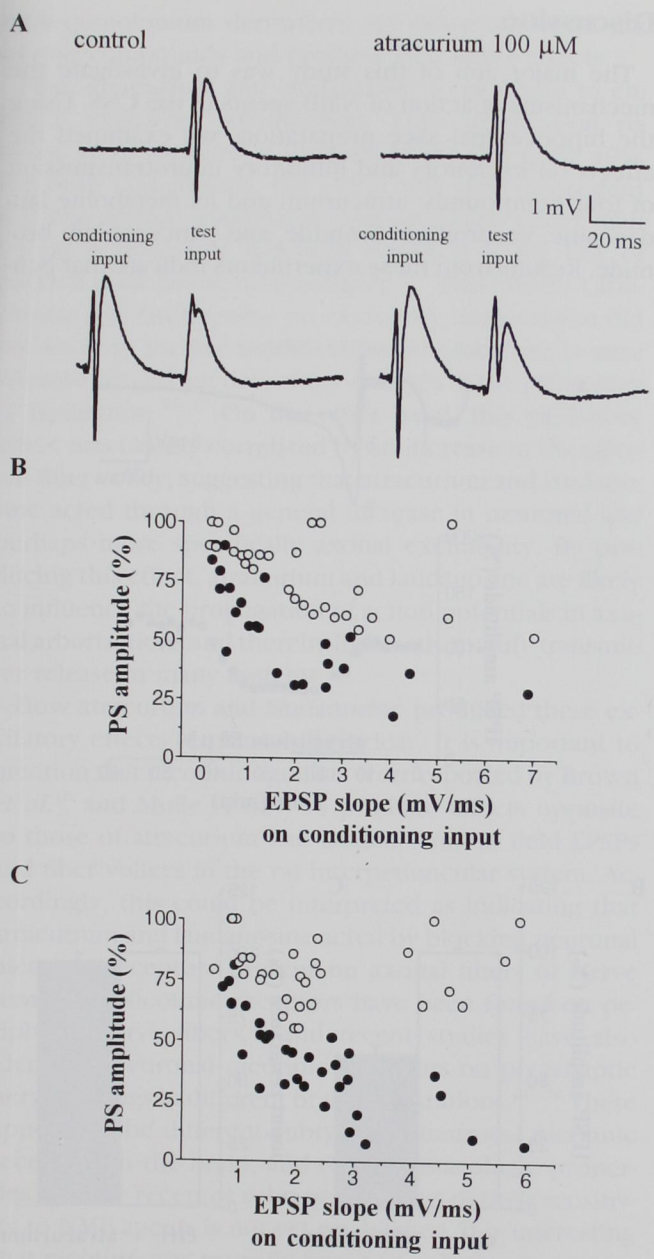

Fig. 5. Blockade of inhibitory mechanisms by atracurium and laudanosine in the hippocampal slice. (*A*) Illustration of field responses recorded in the stratum pyramidale and evoked using a paired-pulse protocol. Traces illustrate the PS elicited on the test input with and without previous activation of the conditioning pathway under control conditions (left) or during atracurium application (right). (*B*) Graph representing in percent the size of the PS elicited on the test input as a function of the size of the synaptic response evoked on the conditioning pathway. Filled circles represent control values and open circles, those obtained during application of 100 μM atracurium. Stimulations of increasing intensity applied to the conditioning pathway generated larger responses and resulted in a progressive elimination of the PS elicited on the test input. In the presence of atracurium, this inhibition of the PS was considerably reduced. Data from six different experiments were pooled. (*C*) Same as in *B* but before and during application of 100 μM laudanosine (n = 10).

pyramidal neurones, intracellular recording techniques were used. Different cellular parameters such as input resistance and size of GABA receptor-mediated synaptic potentials in CA1 pyramidal cells were monitored before and during application of atracurium. Inhibitory postsynaptic potentials (IPSPs) were evoked by orthodromic stimulation of Schaffer collaterals and measured at −60 mV. Under these conditions, afferent stimulation resulted in a short and small EPSP, followed by a long-lasting hyperpolarization mediated by synaptic activation of $GABA_A$ and then $GABA_B$ receptors (fig. 7A, *top trace*). As illustrated in figure 7A, perfusion of 50 μM atracurium for 15 min resulted in a significant and reversible reduction of this GABA receptor-mediated hyperpolarization. Interestingly, the effect was predominantly observed on the first $GABA_A$-mediated component of the IPSP (blockade of 40.5 ± 3.4%; $P < 0.001$; n = 6; measures made at the peak of the IPSP; fig. 7B), with little, if any, effect on the later $GABA_B$-mediated phases of the response (0.4 ± 4.5%; n = 6; measures made 150 ms after stimulation). This inhibition was not accompanied by any significant changes of the input resistance (n = 6; fig. 7C) or of the resting membrane potential. Cells included in this study had a mean resting membrane potential of −58 mV and an input resistance varying between 25–80 MΩ.

These effects of atracurium on inhibitory transmission were obtained for concentrations larger than 1 μM and apparently not for lower concentrations. To confirm this and to analyze this aspect more quantitatively at different concentrations, we carried out experiments in which the $GABA_A$-mediated signal was studied extracellularly in isolation by recording responses evoked in the presence of the glutamate receptor antagonists CNQX (10 μM) and D-AP5 (50 μM). Under these conditions, activation of Schaffer collaterals in the vicinity of the recording pipette evoked a synaptic response exclusively mediated by activation of $GABA_A$ receptors because it could be completely antagonized by bicuculline (10 μM). Application of atracurium at a concentration of 10 nM produced no effect on this inhibitory potential, whereas concentrations of 10 μM reduced

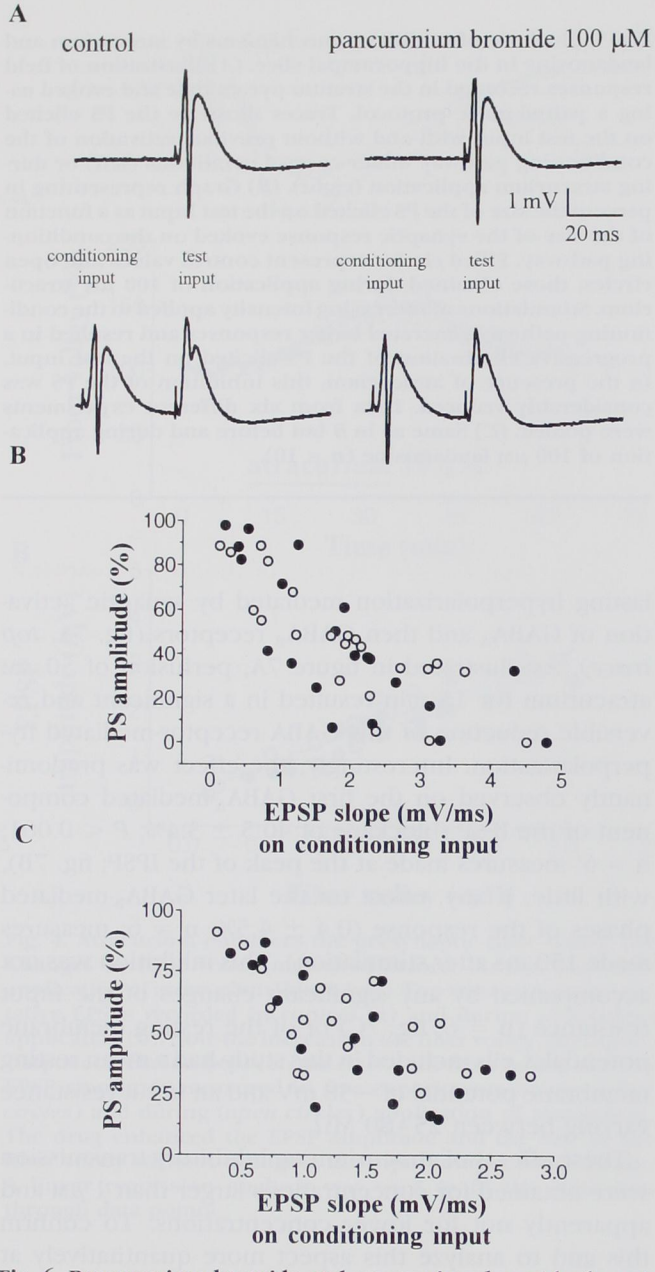

Fig. 6. Pancuronium bromide and vecuronium bromide do not affect PS inhibition. (*A*) Representative field responses recorded in the stratum pyramidale of CA1 and evoked using the same protocol as that described in figure 5. (*B*) Graph representing in percent the size of the PS elicited on the test input as a function of the size of the synaptic response evoked on the conditioning pathway before (*filled circles*) and during (*open circles*) application of 100 μM pancuronium bromide (n = 5). (*C*) Same as in *B* but before (*filled circles*) and during (*open circles*) application of 100 μM vecuronium bromide (n = 5).

this $GABA_A$-mediated response by 32.0 ± 8.0% ($P < 0.001$; n = 4; see table 1). Similarly, laudanosine blocked this inhibitory GABAergic potential only at a concentration of 10 μM (table 1).

Discussion

The major aim of this study was to investigate the mechanisms of action of NMB agents in the CNS. Using the hippocampal slice preparation, we examined the effects on excitatory and inhibitory neurotransmission of four compounds: atracurium and its metabolite laudanosine, vecuronium bromide, and pancuronium bromide. Results from these experiments indicate that ben-

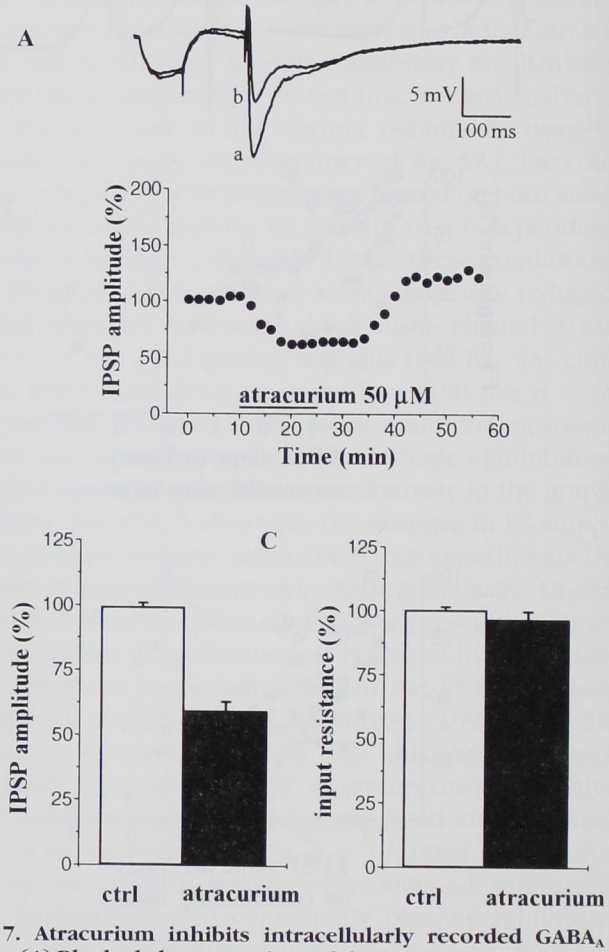

Fig. 7. Atracurium inhibits intracellularly recorded $GABA_A$ IPSPs. (*A*) Blockade by atracurium of the $GABA_A$-mediated IPSP evoked by orthodromic stimulation of Schaffer collaterals. The cell input resistance was assessed by measuring the voltage deflection elicited by a short pulse of hyperpolarizing current (0.2 nA). The graph shows the amplitude of the IPSP measured at the peak of the hyperpolarization before, during, and after atracurium application (*black bar*). Values from eight consecutive measurements were averaged and expressed as percent of baseline pre-drug values. Top traces are representative potentials (average of six) recorded before (a) and during (b) atracurium application. (*B* and *C*) Bar graphs showing respectively, the changes in size of the intracellular IPSP and in input resistance observed in a group of six similar experiments (ctrl = control).

zylisoquinolonium derivatives act differently from the steroid compounds and produce two major effects.

First, atracurium and laudanosine were found to enhance excitatory transmission. Their application resulted in an increase of the field EPSPs averaging 40%. For both compounds, the effect was dose-dependent and was already maximal at concentrations larger than 10 nM, a value that has been reported in the CSF of patients undergoing neurosurgery.[3-5] This effect of atracurium and laudanosine on excitatory transmission did not seem to involve modifications of glutamate release because we did not detect any changes in the properties of facilitation.[16-17] On the other hand, this excitatory effect was closely correlated by an increase in the afferent fiber volley, suggesting that atracurium and laudanosine acted through a general increase in neuronal and perhaps more specifically axonal excitability. By producing this effect, atracurium and laudanosine are likely to influence the propagation of action potentials in axonal arborizations and thereby indirectly modify transmitter release in many systems.

How atracurium and laudanosine produced these excitatory effects remains still unclear. It is important to mention that nicotinic agonists were reported by Brown *et al.*[18] and Mulle *et al.*[19] to produce effects opposite to those of atracurium and laudanosine on field EPSPs and fiber volleys in the rat interpeduncular system. Accordingly, this could be interpreted as indicating that atracurium and laudanosine acted by blocking neuronal nicotinic receptors present on axonal fibers or nerve terminals. Nicotinic receptors have been found on peripheral nerve fibers,[20] and recent studies have also identified neuronal nicotinic receptors on presynaptic nerve endings in different brain preparations.[21-22] There appears to be different subtypes of neuronal nicotinic receptors in the brain, and the pharmacologic properties of these receptor subtypes in terms of their sensitivity to NMB agents is not yet established. It is interesting that nicotine was recently found to enhance excitatory transmission in the hippocampus, probably through an action on neuronal nicotinic receptors containing the $\alpha 7$ subunit.[23] Whether atracurium and laudanosine also acted on this receptor remains unclear because it would be expected either that the presynaptic effect of nicotine was produced through a mechanism of desensitization or that atracurium and laudanosine acted as partial agonists of this receptor, a possibility that cannot be completely excluded.

The second effect of atracurium and laudanosine was to block inhibitory mechanisms in the hippocampal slice. Using a heterosynaptic paired-pulse protocol, we observed that atracurium and laudanosine reversibly decreased the inhibitory effect produced by a conditioning stimulation on the PS evoked 30 ms later on a second independent pathway. However, the concentrations required to reduce inhibition were about one or two orders of magnitude larger than those to enhance excitatory transmission. Therefore, this suggests that atracurium and laudanosine are less potent in reducing inhibition that in enhancing excitatory transmission, indicating that the two actions are likely to involve different receptors or different cellular mechanisms. Intracellular experiments were carried out to determine if the reduction of the inhibitory effect produced by heterosynaptic stimulation resulted from a blockade of GABA transmission. Under these conditions, atracurium was found to block by 40% the size of the hyperpolarizing $GABA_A$-mediated potential evoked by orthodromic stimulation of Schaffer collaterals, whereas the $GABA_B$ component was unaffected. Laudanosine produced similar effects. These observations are thus consistent with the results obtained in previous studies showing that d-tubocurarine increased neuronal excitability and blocked GABAergic transmission.[11,12] Note that nicotine also has been reported to block GABAergic inhibition.[24,25] These effects could thus contribute to the seizure-promoting action of atracurium and laudanosine, although the high concentrations required to block inhibition are less likely to be found in the CSF of patients (see however reference 3).

The mechanisms underlying the blockade of GABAergic inhibition remain unclear. Evidence for the existence of presynaptic nicotinic receptors on GABAergic terminals has been reported.[26-28] There is also evidence that activation of these presynaptic receptors may result in an enhancement of GABA release.[29,26] NMB agents could thus reduce inhibitory mechanisms by interfering with the function of these presynaptic nicotinic receptors on GABAergic terminals. However, the observation of a differential blockade of the $GABA_A$ and $GABA_B$ components of the IPSP suggests that atracurium and laudanosine could rather act by directly influencing the function of $GABA_A$ receptors. This possibility was recently proposed for nicotine[25] and cannot be excluded in the case of atracurium and laudanosine, considering the level of homology existing between nicotinic and GABAergic receptors.[30] Finally, an interference between atracurium and $GABA_A$ receptor function also may occur indirectly. Activation of nicotinic receptors has been shown to result in calcium influx in central

neurones and, through a yet unknown mechanism, inhibition of $GABA_A$ receptor function.[14,31,32] Whatever the explanation, one of the striking aspect of the results presented previously is that atracurium and laudanosine appear to produce effects in the hippocampus that are similar to those induced by nicotine. Whether this is because nicotine application leads to a fast desensitization of nicotinic receptors or because atracurium and laudanosine act as partial agonists of these central receptors will have to be determined.

Finally, it is important to emphasize the contrast observed between the effects of atracurium and laudanosine and those of pancuronium bromide and vecuronium bromide. None of the effects reported previously for atracurium and laudanosine were observed with pancuronium bromide, vecuronium bromide, or its major metabolite 3-OH-vecuronium. A similar difference between these two classes of NMB agents was also reported concerning their effects on calcium influx in hippocampal neurons.[14] This strongly supports the interpretation that the central effects of these two categories of NMB agents do not involve similar mechanisms, although both promote neuronal excitability. From a clinical point of view, this may have interesting implications given that atracurium produced a maximal enhancement of glutamatergic transmission at concentrations around 10 nM, concentrations comparable with those found in the CSF of neurosurgical patients (4–50 ng/ml in a preliminary study on 15 patients[33]). This is certainly an important aspect to consider, given the increasing evidence that conditions associated with a passage of NMB agents into the CNS are more numerous than initially thought.[34] It is thus likely that the excitatory effects reported here on glutamatergic transmission are relevant in terms of clinical side effects produced by these NMB agents during neurosurgery or in intensive care patients.

References

1. Smith SM, Brown HO, Toman JEP, Goodman LS: The lack of cerebral effects of *d*-tubocurarine. ANESTHESIOLOGY 1947; 8:1–14

2. Matteo RS, Pua EK, Khambatta HJ, Spector S: Cerebrospinal fluid levels of *d*-tubocurarine in man. ANESTHESIOLOGY 1977; 46:396–9

3. Eddleston JM, Harper NJN, Pollard BJ, Edwards D, Gwinnutt CL: Concentrations of atracurium and laudanosine in cerebrospinal fluid and plasma during intracranial surgery. Br J Anaesth 1989; 63:525–30

4. Fahey MR, Claver Canfell P, Taboada T, Hosobuchi Y, Miller RD: Cerebrospinal fluid concentrations of laudanosine after administration of atracurium. Br J Anaesth 1990; 64:105–6

5. Miller RD: Muscle relaxants in the ICU. ASA 1994 Annual Refresher Course Lectures, San Francisco, 1994; 255–73

6. Dasheiff RM: *d*-Tubocurarine causes neuronal death when injected directly into rat brain. Exp Neurol 1985; 89:172–88

7. Goonewardene TW, Sentheshanmuganathan S, Kamalanathan S, Kanagasunderam R: Accidental subarachnoid injection of gallamine: a case report. Br J Anaesth 1975; 47:889–93

8. Mesry S, Baradaran J: Accidental intrathecal injection of gallamine triethiodide. Anaesthesia 1974; 29:301–4

9. Peduto VA, Gungui P, Di Martino MR, Napoleone M: Accidental subarachnoid injection of pancuronium. Anesth Analg 1989; 69:516–7

10. Szenohradszky J, Trevor AJ, Bickler P, Caldwell JE, Sharma ML, Rampil IJ, Miller RD: Central nervous system effects of intrathecal muscle relaxants in rats. Anesth Analg 1993; 76:1304–9

11. Hill RG, Simmonds MA, Straughan DW: Convulsive properties of *d*-tubocurarine and cortical inhibition. Nature (Lond) 1972; 240:51–2

12. Lebeda FJ, Hablitz JJ, Johnston D: Antagonism of GABA-mediated responses by *d*-tubocurarine in hippocampal neurons. J Neurophysiol 1982; 48:622–32

13. Vernino S, Amador M, Luetje CW, Patrick J, Dani JA: Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. Neuron 1992; 8:127–34

14. Cardone C, Szenohradszky J, Yost S, Bickler PE: Activation of brain acetylcholine receptors by neuromuscular blocking drugs. ANESTHESIOLOGY 1994; 80:1155–61

15. Lambert NA, Borroni AM, Grover LM, Teyler TJ: Hyperpolarizing and depolarizing $GABA_A$ receptor-mediated dendritic inhibition in area CA1 of the rat hippocampus. J Neurophysiol 1991; 66:1538–48

16. McNaughton BL: Long-term synaptic enhancement and short-term potentiation in rat fascia dentata act through different mechanisms. J Physiol (Lond) 1982; 324:249–62

17. Muller D, Lynch G: Evidence that changes in presynaptic calcium currents are not responsible for long-term potentiation in hippocampus. Brain Res 1989; 479:290–9

18. Brown DA, Docherty RJ, Halliwell J: The action of cholinomimetic substances on impulse conduction in the habenulo-interpeduncular pathway of the rat *in vitro*. J Physiol (Lond) 1984; 353:101–9

19. Mulle C, Vidal C, Benoit P, Changeux JP: Existence of different subtypes of nicotinic acetylcholine receptors in the rat habenulo-interpeduncular system. J Neurosci 1991; 11:2588–97

20. Armett CJ, Ritchie JM: The action of acetylcholine on conduction in mammalian non-myelinated fibres and its prevention by anticholinesterase. J Physiol (Lond) 1960; 152:141–58

21. Bertrand D, Changeux JP: Nicotinic receptor: an allosteric protein specialized for intercellular communication. Sem Neurosci 1995; 7:75–90

22. McGehee D, Role LW: Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. Annu Rev Physiol 1995; 57:521–46

23. Gray R, Rajan AS, Radcliffe KA, Yakehiro M, Dani JA: Hippocampal synaptic transmission enhanced by low concentrations of nicotine. Nature 1996; 383:713–6

24. Freund RK, Luntz-Leybdman V, Collins AC: Nicotine interferes with GABA-mediated inhibitory processes in mouse hippocampus. Brain Res 1990; 527:286–91

25. Wotring VE, Yoon KW: The inhibitory effects of nicotinic antagonists on currents elicited by GABA in rat hippocampal neurons. Neuroscience 1995; 67:293–300

26. Léna C, Changeux JP, Mulle C: Evidence for "preterminal" nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. J Neurosci 1993; 13:2680–8

27. Yang X, Criswell HE, Breese GR: Nicotine-induced inhibition in medial septum involves activation of presynaptic nicotinic cholinergic receptors on γ-aminobutyric acid-containing neurons. J Pharmacol Exp Ther 1996; 276:482–9

28. Léna C, Changeux JP: Role of Ca^{2+} ions in nicotinic facilitation of GABA release in mouse thalamus. J Neurosci 1997; 17:576–85

29. King WM: Nicotinic depolarization of optic nerve terminals augments synaptic transmission. Brain Res 1990; 527:150–4

30. Galzi JI, Changeux JP: Neuronal nicotinic receptors: Molecular organization and regulations. Neuropharmacology 1995; 34:563–82

31. Mulle C, Choquet D, Korn H, Changeux JP: Calcium influx through nicotinic receptor in rat central neurons: Its relevance to cellular regulation. Neuron 1992; 8:135–43

32. Albuquerque EX, Pereira EFR, Castro NG and Alkondon M: Neuronal nicotinic receptors: Function, modulation and structure. Sem Neurosci 1995; 7:91–101

33. Tassonyi E, Fuchs-Buder T, Chiodini F, Muller D, Bertrand D, Fathi M: Cerebrospinal fluid concentrations of atracurium in neurosurgical patients [Abstract]. Br J Anaesth 1997; 78:A246

34. Friedman A, Kaufer D, Shemer J, Hendler I, Soreq H and Tur-Kaspa I: Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. Nature Med 1996; 2:1382–5