Background: Curaremimetic nondepolarizing muscle relaxants are widely used in clinical practice to prevent muscle contraction either during surgery or during intensive care. Although primarily acting at the neuromuscular junction, these compounds can cause adverse effects, including modification of cardiac rhythm, arterial blood pressure, and in the worst cases, triggering of seizures. In this study, we assessed the interaction of atracurium and its metabolite, laudanosine, with neuronal nicotinic receptors.

Methods: The human neuronal nicotinic receptors α4β2, α3β4, α3α5β4, and α7 are heterologously expressed in Xenopus laevis oocytes, and the effect of atracurium and its degradation product, laudanosine, were studied on these receptors.

Results: Atracurium and laudanosine inhibited in the micromolar range the major brain αβ2 receptor and the ganglionic α3β4 or α3β4δ5 and the homomeric α7 receptors. For all four receptors, inhibition was rapid and readily reversible within less than 1 min. Atracurium blockade was competitive at αβ2 and α7 receptors but displayed a noncompetitive blockade at the α3β4 receptors. Inhibition at this receptor subtype was not modified by α5. Laudanosine was found to have a dual mode of action; first, it competes with acetylcholine and, second, it blocks the ionic pore by ionic hindrance. At low concentrations, these two drugs are able to activate both the αβ2 and the α3β4 receptors.

Conclusion: Adverse effects observed during atracurium administration may be attributed, at least partly, to an interaction with neuronal nicotinic receptors.

THE benzylisoquinoline derivative atracurium (ATR) is a widely used nondepolarizing neuromuscular blocking agent, with a relatively short half-life of 20–40 min, and a rapid elimination period.2–4 Although well-tolerated, atracurium can cause different adverse reactions,5,6 such as cardiovascular effects, that are thought to be mediated by the ganglionic neuronal nicotinic acetylcholine receptor (nAChR).7 In addition, atracurium and its degradation product laudanosine were found in cerebrospinal fluid, indicating that this compound may cross, in some circumstances, the blood–brain barrier.8,9 and could, depending on the concentrations, affect brain function. In agreement with this hypothesis, it has been shown in animal models that, when administered at high concentration, neuromuscular blocking agents can trigger seizures.10,11 Furthermore, atracurium application to hippocampal slices modifies synaptic transmission.12

The recent availability of complementary DNA (cDNA) coding for human neuronal nAChRs (reviewed in Bertrand and Changeux13 and Lindstrom et al.,14) opens new possibilities for assessment of the possible effects of atracurium on these receptors. The aim of this study was to evaluate the effects of atracurium and laudanosine on the functional properties of the major brain and ganglionic human nAChRs reconstituted in Xenopus oocytes. The α4β2 subunits, which are thought to constitute the major brain nicotinic receptors, were chosen as a model of central nAChRs.13,14 Receptors corresponding to those found in ganglia were obtained by expression of α3β4 or α3β4α5 subunits.15 In addition, we evaluated atracurium and laudanosine effects on the homomeric α7 nAChR that is expressed centrally and peripherally.13,16–18

Methods

Oocyte Preparation and cDNA Injection

Xenopus oocytes were isolated and prepared as previously described.19 Oocytes were intranuclearly injected with 2 ng cDNA. All subunits were injected with an equal concentration. Oocytes were kept separately in a 96-well microtiter plate (NUNC) at 18°C in Barth solution (88 mM NaCl, 1 mM KCl, 2.4 mM NaHCO3, 10 mM HEPES, 0.82 mM MgSO4·7H2O, 0.33 mM Ca(NO3)2·4H2O, 0.41 mM CaCl2·6H2O, at pH 7.4 adjusted with NaOH, and supplemented with 20 μg/ml kanamycine, 100 U/ml penicillin, and 100 μg/ml streptomycine). Atracurium (Tracrium®) was purchased from Glaxo Wellcome (London, UK). All other drugs, including ACh, laudanosine and atropine were purchased from Sigma (Buchs, Switzerland).

Electrophysiology

Current recordings from oocytes were performed at 18°C, 2–4 days after cDNA injections. During the recording, cells were continuously superfused with original Ringer 2 (82.5 mM NaCl, 2.5 mM KCl, 5 mM HEPES, pH

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As shown in figure 1A, the application of 10 μM atracurium markedly inhibits the ACh-evoked current at the α4β2 nAChR. This effect was reversible within 1 min of washout. A comparable inhibition of ACh-evoked current was observed at 4 μM atracurium for the ganglionic α3β4 and α3β4α5 nAChRs and at 10 μM atracurium for the α7 receptor. Full recovery was observed within 2 min. For each of these receptor subtypes, the ACh concentration test pulse was adjusted to near their respective EC50. A small inward deflection of the current was observed during atracurium application alone on the α4β2 and α3β4 receptors. To further evaluate this putative receptor activation, currents evoked by low ACh concentrations were compared with those evoked by either atracurium or laudanosine alone (fig. 1B). Increasing the drug concentrations greater than those shown always resulted in a smaller current, indicating that these compounds act as inhibitors at relatively higher concentrations. The comparison of the currents evoked by ACh, atracurium, and laudanosine on a log-log scale, highlights the differences in sensitivity of the α3β2 and α3β4 receptors to these three substances, with ACh always being the most effective agonist.

As shown in figure 1B, data are well-fitted by straight lines and yielded respective slope values of 0.73 and 0.78 for ACh and atracurium on the α4β2 and 1.22, 0.6, and 0.31 for ACh, laudanosine, and atracurium on the α3β4. Offset values were 2.72 and 2.3 for ACh and atracurium on the α4β2 and 1.5, 0.56, and −0.02 for ACh, laudanosine, and atracurium on the α3β4. All correlation factors were superior to 0.95. As expected for a receptor with a lower affinity, all responses on the α3β4 are shifted to the right. Therefore, the relative agonist sensitivities are ACh greater than atracurium on α4β2 receptors and ACh greater than laudanosine greater than atracurium on α3β4 receptors. Laudanosine alone, however, evoked no detectable current at α4β2. Similarly, no currents could be recorded in response to atracurium or laudanosine exposure at the homomeric α7 receptors (data not shown). Given the paucity of α5 expression and the absence of distinguishable atracurium effects, no attempts were made to characterize the activation of this receptor subtype.

**Dual Blockade Mechanisms of Atracurium**

To further characterize the atracurium inhibition, we first evaluated the atracurium concentration–response inhibition curve at a fixed ACh test condition (fig. 2 and table 1). Afterward, atracurium was kept at a constant concentration while the concentration of ACh was progressively increased. As shown in figure 2A, IC50 of the α4β2 nAChR was observed at 1.5 μM atracurium, for 0.1 μM ACh test pulse. Increasing the ACh concentration decreased the IC50 value, suggesting that atracurium and ACh may compete for the same binding site at α4β2. The competitive nature of atracurium blockade at the α4β2...
nAChR was further confirmed with the evaluation of how atracurium altered the ACh concentration–response relation (fig. 2B). For each cell, data were normalized to the saturating current recorded at maximal ACh concentration (1 mM) in control conditions. The graph shows that 10 μM atracurium caused a shift of the concentration–response curve toward higher concentrations (table 1), without affecting the maximal evoked current.

As shown in figures 2C and 2D, a different pattern of inhibition was observed at the ganglionic α3β4 receptor. First, inhibition was independent of the ACh concentration (fig. 2C). Second, atracurium caused only a small decrease on the ACh sensitivity, and blockade could not be relieved by increasing the ACh concentration. Because it is known that α5 receptor contributes to a fraction of ganglionic receptors, the effects of atracurium were assessed after coinjection of α3, β4, and α5 subunits. As shown in table 1, injection of this subunit caused no detectable changes in atracurium affinity. No differences could be observed on the ACh concentration–response profile either (data not shown). These results suggest that atracurium may act on the ganglionic receptor as an open channel blocker.
Quantification of the atracurium inhibition at the $\alpha^7$ receptor with three ACh test pulse conditions indicates that, as for the $\alpha^4\beta^2$ nAChR, the IC$_{50}$ progressively shifted toward the lower sensitivities as the agonist concentration was increased (fig. 2E, table 1). The IC$_{50}$ dependency of the ACh concentration is indicative of the competitive mode of action of atracurium. This hypothesis was further reinforced by the observation that atracurium inhibition is fully overcome by an increase of the ACh concentration (fig. 2F). Because $\alpha^7$ is highly permeable to Ca$^{2+}$ (its response may be contaminated by calcium-dependent chloride activation), atracurium concentration–response inhibitions were measured in a Ba$^{2+}$-containing medium, a condition that is known to reduce chloride activation. Substitution of extracellular calcium by barium caused a small shift to the left of the IC$_{50}$ and slightly increased the EC$_{50}$ (300–430 $\mu$M, data not show). This indicates that, even when present, chloride contamination plays a minor role in the atracurium blockade. The lower calcium permeability of $\alpha^4\beta^2$ or $\alpha^3\beta^4$ receptors would imply that calcium-dependent chloride contamination might also be neglected for these subtypes. Therefore, all further experiments were performed during normal divalent cation conditions.

**Effects of Laudanosine on the Central or Ganglionic nAChRs**

To isolate the effects of atracurium from those of laudanosine, experiments were performed using pure laudanosine. When the same experimental protocols as those presented in figure 2 were used, we found that...
Acetylcholine (ACh) concentration of half activation (EC50) measured in control and during atracurium exposures (10 or 5 μM) are given. Mean and SD values corresponding to the best fits obtained with the empirical equation (1) are indicated. Atracurium concentration–response inhibition curves, measured as in figure 2A, were characterized with their respective concentration of half inhibition (IC50) values. Mean and SD values obtained by curve-fitting with the Hill equation (2) are indicated. Numbers in parentheses indicate the number of cells tested in each condition. No statistical difference was observed in EC50, IC50, and Hill coefficient between α3β4 and α3β4α5.

cDNA = complementary DNA; nACHR = neuronal nicotinic acetylcholine receptor; nH = apparent cooperativity.

Table 1. Effects of Atracurium at α4β2, α3β4, α3β4α5, and α7 nACHRs

<table>
<thead>
<tr>
<th>Human cDNA</th>
<th>[ACh] (μM)</th>
<th>IC50 (μM)</th>
<th>nH</th>
</tr>
</thead>
<tbody>
<tr>
<td>α4β2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 8)</td>
<td>1.8 ± 1.2</td>
<td>0.97 ± 0.16</td>
<td>0.1 (n = 17)</td>
</tr>
<tr>
<td>10 μM Atracurium (n = 8)</td>
<td>5.8 ± 4.1</td>
<td>0.87 ± 0.38</td>
<td>1 (n = 7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α3β4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 11)</td>
<td>56.8 ± 2.75</td>
<td>1.75 ± 0.04</td>
<td>10 (n = 6)</td>
</tr>
<tr>
<td>5 μM Atracurium (n = 7)</td>
<td>67.6 ± 2.43</td>
<td>1.73 ± 0.03</td>
<td>50 (n = 7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α3β4α5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 12)</td>
<td>51.8 ± 5.70</td>
<td>1.62 ± 0.08</td>
<td>10 (n = 6)</td>
</tr>
<tr>
<td>5 μM Atracurium (n = 7)</td>
<td>85.8 ± 10.3</td>
<td>1.61 ± 0.05</td>
<td>50 (n = 7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>108 ± 17.9</td>
<td>1.56 ± 0.13</td>
<td>30 (n = 7)</td>
</tr>
<tr>
<td>10 μM Atracurium (n = 5)</td>
<td>284 ± 66.4</td>
<td>1.56 ± 0.13</td>
<td>100 (n = 15)</td>
</tr>
</tbody>
</table>

Atracurium also inhibits the α4β2, α3β4, and α7 receptors (fig. 3, table 2).

Measurement of the fraction of ACh current inhibition at α4β2 as a function of agonist concentration showed that laudanosine blockade was only partially removed by increasing the ACh concentration (fig. 3A). As for atracurium, adequate curve fitting was obtained with the Hill equation (equation 1), providing addition of a scaling factor of 0.65 to account for the laudanosine insurmountable blockade. These data show that, in contrast to atracurium, the mechanism of laudanosine blockade on the α4β2 receptor is competitive, but this compound acts also in a noncompetitive manner for at least 35% of the blockade. Typical ACh-evoked currents recorded in control and during coapplication of laudanosine, are shown in figure 3B. Contrary to atracurium, laudanosine alone caused no detectable signal. The small rebound observed at the end of the ACh-laudanosine application is compatible with mechanisms of open channel blockade. Concentration–response inhibition measured with a 0.1-μM ACh test pulse yielded an IC50 of 9.4 μM (table 2). Here we used low ACh concentration to avoid rapid desensitization of this receptor.

A difference in the mode of action between atracurium and laudanosine was also identified at the ganglionic α3β4 nACHR (figs. 3C and D). Figure 3C shows the dual mode of blockade caused by laudanosine with a shift in the ACh EC50 (table 2) and an insurmountable blockade. Another difference was the inward currents observed during the prepulse of laudanosine alone (figs. 1B and 3D). An important rebound of current was observed at the end of the ACh–laudanosine application (fig. 3D). This rebound was observed in every cell tested. Concentration–response curves to laudanosine yielded an IC50 of approximately 38 μM for an ACh test pulse of 50 μM (table 2).

A dual mode of action of laudanosine was also observed on the α7 receptor but with a smaller fraction of insurmountable blockade (fig. 3E). The Hill equation was used with a scaling factor of 0.85, introduced into the curve fitting to account for this small fraction of blockade. The ACh EC50 increased from 80 to 240 μM during exposure to 30 μM laudanosine (table 2). It is well-documented that when charged molecules enter and block the ionic pores of a ligand-gated channel, its fraction of blockade will depend on the transmembrane potential. Therefore, if laudanosine causes a blockade by steric hindrance in the channel pore, its inhibition may be voltage dependent. Typical current-voltage relations recorded in control and during laudanosine exposure showed a marked voltage dependency of laudanosine blockade (fig. 3F). The small rebound observed at the end of the ACh–laudanosine application is coherent with a mechanism of open channel blockade. Determination of the concentration–response inhibition profile with an ACh test pulse of a 100 μM yielded an IC50 of 18.3 μM (table 2).

**Discussion**

Recent advances in molecular biology and DNA cloning have identified the nACHR subtypes expressed in various regions of the central and peripheral nervous system (reviewed in Bertrand and Changeux and Lindstrom et al. Central nicotinic receptors mainly con-
tand the α4 and β2 subunits, whereas ganglionic receptors result from the assembly of α3 and β4. Coimmunoprecipitation experiments have shown that a fraction of ganglionic receptors also contain the α5 subunit. Finally, it has been shown that the homomeric α7 receptor is expressed centrally and peripherally. To evaluate the possible interaction between neuronal nAChRs and atracurium and its first degradation product laudanosine, these four receptor subtypes were applied alone or with ACh on *Xenopus* oocytes expressing the human α4β2, α3β4, and α5, and α7 receptors.

Incubation with atracurium or laudanosine caused a marked inhibition of these four receptor subtypes, with IC50 in the micromolar range (tables 1 and 2). Blockade of the ACh-evoked current was fast, and complete recovery was obtained within 1 min, indicating rapid onset and offset kinetics. In addition, as expected from data obtained on muscle nAChRs, atracurium and laudanosine effects are multiple: (1) competitive inhibition, (2) open channel blockade, and (3) activation of the receptor. The latter effect was observed only for the central α4β2 and ganglionic α3β4 receptors. It is noteworthy to recall that the α5 subunit does not contribute to the pharmacologic profile of the ganglionic receptor. Therefore, it is not surprising that injection of this subunit caused no detectable changes in atracurium sensitivity of the α3β4 receptor.

One of the difficulties in the study of the effects caused by atracurium is the instability of this product. It is well-documented that one molecule of atracurium quickly degrades into two laudanosine molecules. The Hoffman degradation of atracurium is independent of
biologic processes and therefore precludes an evaluation of the effects of atracurium alone. A few percent contamination of laudanosine in the atracurium solution cannot be excluded. A comparison of the atracurium and laudanosine effects is mandatory to understand their respective contribution. As shown in figures 2 and 3, where atracurium and laudanosine caused a comparable inhibition, a marked difference between these two compounds was observed when comparing the fraction of current they can activate. The major brain \( \alpha \beta \delta \beta \) nACHR was activated by low atracurium concentrations but was unresponsive to laudanosine (data not shown). In contrast, the ganglionic \( \alpha \beta \beta \delta \) nACHR was activated by laudanosine but almost unresponsive to atracurium. Plots of the ACh, atracurium, and laudanosine currents evoked by low concentrations of these three compounds on a log-log scale show that neuronal nACHRs are at least five to fifty times more sensitive to ACh than to curaremimetics. At higher concentrations, atracurium and laudanosine inhibit the ACh-evoked currents. In the absence of significant differences between the \( \alpha \beta \beta \delta \) and the \( \alpha \beta \beta \delta \alpha \delta \) receptor profiles and given the paucity of expression of these latter receptor subtypes, activation experiments were therefore not performed.

Plasma levels of atracurium range between 0.5 and 5.1 \( \mu \)g/ml (0.4 and 4.1 \( \mu \)M, respectively), with the lowest values found during surgical anaesthesia and the highest during intensive care conditions. The degradation of one atracurium molecule into two laudanosine molecules implies that a plasma concentration as high as 8 \( \mu \)M, respectively, when stimulated with M ACh (tables 1 and 2). The ACh EC\(_{50}\) was shifted toward higher concentration in the presence of a constant atracurium concentration (figs. 2B and 3B). The atracurium blockade was fully reversible by increasing the ACh concentration, whereas an insurmountable block of approximately 35% persisted with laudanosine. This indicates differences in the mode of action, with these two compounds atracurium inducing a purely competitive blockade and laudanosine a mixed competitive and noncompetitive action. These data are in agreement with previous findings that showed that \( \alpha \)tubocurarine, a related chemical structure, is a competitive inhibitor on the chick \( \alpha \beta \beta \delta \) nACHR with an IC\(_{50}\) in the micromolar range.\(^{\text{31}}\) Evidence for open channel blockade is clearly seen with the rebounds observed at the end of ACh and laudanosine coapplication on the \( \alpha \beta \beta \delta \) receptor (fig. 3B). The difference observed in EC\(_{50}\) for the \( \alpha \beta \beta \delta \) receptor between tables 1 and 2 is attributable to the use of different oocyte batches. A recent report showed that the neuronal nACHR concentration-response curves are best fitted using the two Hill equations.\(^{\text{32}}\) However, because of technical limitations, the number of points collected was restricted and does not allow for further conclusion.

Muscle relaxant drugs have been described to have adverse effects and, in the worst cases, can trigger seizures in vitro or in the animal model.\(^{\text{10,11}}\) Here we report that atracurium and laudanosine can block the major brain nicotinic receptor at concentrations that can be present in the plasma of patients. Recently mutations on the \( \alpha \) subunit have been shown to induce autosomal dominant nocturnal epilepsy (reviewed in Steinlein\(^ {\text{33}}\)). It follows that a modification of the \( \alpha \beta \beta \delta \) receptor activity could be the origin of seizures. Even if these receptors are exclusively expressed in the brain and therefore protected by the blood-brain barrier, it has been already shown that atracurium and laudanosine can be found in cerebrospinal fluid.\(^{\text{8,9}}\) This suggests that part of atracurium adverse effects may be caused through its action on the \( \alpha \beta \beta \delta \) receptor.

A different mode of action was observed at the ganglionic \( \alpha \beta \beta \delta \) nACHR with atracurium and laudanosine, causing a noncompetitive blockade. Note that, in agreement with the absence of effects of \( \alpha \delta \) on ganglionic receptor pharmacology, the addition of \( \alpha \delta \) caused no

| Table 2. Effects of Laudanosine on \( \alpha\beta\delta\beta \), \( \alpha\beta\beta\delta \), and \( \alpha\delta \) nACHRs |
|-----------------|------------|------------|-------------|-------------|
| Human cDNA      | \( [\text{EC}_{50}] \) (\( \mu \)M) | nH         | \( [\text{ACh}] \) (\( \mu \)M) | \( [\text{IC}_{50}] \) (\( \mu \)M) |
| \( \alpha\beta\delta\beta \) |            |            |             |             |
| Control (n = 6) | 14.1 ± 7.47 | 0.66 ± 0.10 | 0.1 (n = 7) | 9.41 ± 2.23 |
| 100 \( \mu \)M Laudanosine (n = 6) | 65.0 ± 33.46 | 0.68 ± 0.10 | 0.1 (n = 7) | 9.41 ± 2.23 |
| \( \alpha\beta\beta\delta \) |            |            |             |             |
| Control (n = 6) | 36 ± 20.73  | 0.98 ± 0.13 | 50 (n = 8)  | 38.37 ± 15.28 |
| 100 \( \mu \)M Laudanosine (n = 6) | 110 ± 16.33 | 0.97 ± 0.09 | 50 (n = 8)  | 38.37 ± 15.28 |
| \( \alpha\delta \) |            |            |             |             |
| Control (n = 11)| 80.22 ± 14.14 | 1.54 ± 0.16 | 100 (n = 7) | 18.34 ± 5.08 |
| 30 \( \mu \)M Laudanosine (n = 11) | 239.8 ± 86.48 | 1.78 ± 0.27 | 100 (n = 7) | 18.34 ± 5.08 |

This table summarizes the concentration of half activation (\( \text{EC}_{50} \)) and concentration of half inhibition (\( \text{IC}_{50} \)) values obtained as in table 1, but for laudanosine. Values are expressed as mean and SD. The number of cells tested is indicated in parentheses.

cDNA = complementary DNA; nAChR = neuronal nicotinic acetylcholine receptor; nH = apparent cooperativity; ACh = acetylcholine.

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detectable changes on the action of atracurium. The ganglionic α3β4 receptor was inhibited by atracurium and laudanosine with an IC50 of 3.2 and 38.4 μM, respectively. In agreement with a noncompetitive blockade, half-inhibition was independent on the agonist test pulse concentration. Moreover, the addition of a constant inhibitor concentration induced a slight shift of the concentration–response curve toward higher ACh concentrations, but the maximal response measured in the presence of an antagonist was reduced by at least 35%. In addition, for laudanosine, an important rebound indicative of an open channel blockade was observed at the end of the test pulse. Results obtained with atracurium on the α3β4 receptor suggest that this compound blocks these receptors by noncompetitive blockade. Finally, the atracurium IC50 of 3.2 μM observed in our conditions (table 1) is compatible with the 3 μM dissociation constant reported for d-tubocurarine blockade on rat ganglia.54

Recalling that the blood–brain barrier does not isolate autonomic ganglia, it is conceivable that concentrations of atracurium and laudanosine comparable with plasma levels may be reached in their environment. Our data are in agreement with previous hypotheses that adverse cardiovascular effects may be attributed to the direct action of atracurium or laudanosine on cardiac ganglia.7

At the homomeric a7 receptor, atracurium acts as a competitive inhibitor. This was shown by the shift in the IC50 of the atracurium blockade in function of the ACh test pulse concentration and by the full relief of inhibition observed at saturating ACh. Therefore, as for the α4β2 and α3β4, it can be concluded that the effects caused by laudanosine contaminant on a7 are negligible. Previous studies of the blockade caused by d-tubocurarine have shown that this compound acts as a noncompetitive blocker on the chick receptor and that 0.5 μM was already sufficient to reduce by 40% the ACh evoked current.35 Although initially different, these results and ours are not contradictory. The difference in the mode of action between atracurium and d-tubocurarine may be attributed to the difference in size between these two molecules. Moreover, experiments performed with the desensitized open L247T receptor have shown the dual mode of action of d-tubocurarine, with activation and blockade of this mutant.56 Although it was proposed that, in some cases, perfusion conditions might affect the a7 responses in Xenopus oocytes,57 we think that the agreement between our results and those obtained with the same cDNA expressed in human embryonic kidney cells is indicative of adequate experimental conditions. Therefore, no attempts were made to compensate ACh concentration–response curves, and raw data are presented herein.

The a7 receptor is expressed in both the central and the peripheral nervous systems (reviewed in Bertrand and Changeux13 and Lindstrom et al.14), but also in nonneuronal cells such as the embryonic skeletal muscle cells. However, a7 has never been found in adult innervated muscle.38–40 According to these findings, we can conclude that muscular effect observed during atracurium treatment cannot be caused by interaction with a7 receptors. The atracurium and laudanosine IC50 values on this receptor were 3.1 and 18.3 μM, respectively. These data confirmed that atracurium and laudanosine in therapeutic conditions could block a7 receptors. Therefore, we cannot exclude that side effects observed during atracurium administration could be caused by a direct effect on these receptors.

In conclusion, we have shown that atracurium and laudanosine interact with neuronal nicotinic ACh receptors at concentrations that can be present in clinical conditions.

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