Action of Certain Tropine Esters on Voltage-Clamped Lobster Axon

M. P. BLAUSTEIN

From the Naval Medical Research Institute, Bethesda, Maryland 20014. Dr. Blaustein's present address is Department of Physiology, Cambridge University, England

ABSTRACT Tropine p-tolylacetate (TPTA) and its quaternary analogue, tropine p-tolylacetate methiodide (TPTA MeI) decrease the early transient (Na) and late (K) currents in the voltage-clamped lobster giant axon. These agents, which block the nerve action potential, reduce the maximum Na and K conductance increases associated with membrane depolarization. They also slow the rate at which the sodium conductance is increased and shift the (normalized) membrane conductance vs. voltage curves in the direction of depolarization along the voltage axis. All these effects are qualitatively similar to those resulting from the action of procaine on the voltage-clamped axon. One unusual effect of the tropine esters, noticeable particularly at large depolarization steps, is that they cause the late, K current to reach a peak and then fall off with increasing pulse duration. This effect has not been reported to occur as a result of procaine action. Tropine p-chlorophenyl acetate (TPTClA), which differs from TPTA only by the substitution of a p-Cl for a p-CH₃ group on the benzene ring, had a negligible effect on axonal excitability.

INTRODUCTION

The mechanism of local anesthetic action in blocking neural excitation must certainly be closely related to the structure of the anesthetic molecules, and there have been numerous studies of the correlations between molecular structure and local anesthetic activity. In general, as pointed out by Løfgren (1948), most local anesthetic molecules include (a) a lipophilic aromatic ring, separated by (b) an intermediate chain, from (c) a hydrophilic amino group. Most clinically useful anesthetics are secondary or tertiary amines, and are thus able to exist in solution as both the uncharged free base, and as the substituted cation. The question of whether the charged or the uncharged form of these drugs is the active one has been a major point of controversy. This subject has recently been reviewed by Ritchie and Greengard (1966),

1 For the purposes of this discussion, we will arbitrarily define a local anesthetic as an agent which, when applied directly to peripheral nerve, blocks excitability.
who concluded that the cation is the active form of the local anesthetic molecule. Perhaps unfortunately, most of the evidence relevant to this problem comes from studies on the relation between pH and local anesthetic activity. As pointed out by Ritchie and Greengard (1966), interpretation of these data has led to conflicting conclusions partly as a result of the difficulty in distinguishing between a direct effect of the drug molecule at its site of action, and the indirect influence of the rate of drug penetration to its site of action.

A more direct approach to the study of the active form of the drug molecule might be to compare the anesthetic activity of a tertiary amine molecule with its quaternary analogue, since the quaternary amine exists only as a cation. Such an analogue pair, tropine-\(p\)-tolylacetate (TPTA), and tropine-\(p\)-tolylacetate methiodide (TPTA MeI) (see Fig. 1), have recently been synthesized by Friess and his colleagues (1961, 1966 a). These tropine esters have been tested for their effects on the ionic conductances of the voltage-clamped lobster giant axon. As previously reported (Goldman and Blaustein, 1966), TPTA reduces both the early transient and late steady-state conductance increases associated with excitation in nerve. The present communication provides a detailed comparison between the tertiary and quaternary tropine esters. The effects of a third ester, tropine-\(p\)-chlorophenyl acetate (TPCl\(\phi\)A) are also described.

**METHODS**

The sucrose gap-voltage clamp techniques and artificial seawater (buffered at pH 7.4) were the same as those used in the previous studies (Blaustein, 1968; Blaustein and Goldman, 1968).

**RESULTS**

1. **Action of Tropine Esters on the Nerve Action Potential**

The action potentials in Fig. 2 demonstrate that TPTA MeI reversibly reduces the amplitude of the action potential and prolongs its duration. The latter effect is manifested primarily by a marked slowing of the rate of repolariza-
tion. Although not shown here, the effects of TPTA were indistinguishable from those of TPTA MeI, while TPClφA has no significant effect on the action potential.

2. Effect of Tropine Esters on Membrane Current-Voltage Relations

The current vs. time curves in Fig. 3 show that the early transient (Na) and late “steady-state” (K) currents are both blocked reversibly by TPTA.

![Figure 2: Axon L-26-65. T = 7°C. Action potentials before (A), during (B), and after (C) 2.5 mM TPTA MeI.](image)

![Figure 3: Axon L-25-65. T = 5°C. Current-time curves before and during treatment with 2.5 mM TPTA, and during recovery in 2.5 mM TPClφA. At +98 mv the sodium current is buried in the capacity transient. Inward current is downward relative to the zero-current reference at the left of each curve. The 4 sec time calibration refers only to the lower row of current-time curves.](image)

TPClφA, which by itself has no effect on the current-time curves, does not even prevent recovery from treatment with TPTA (Fig. 3) when added to the wash solution. TPTA MeI, like TPTA, reduces both the Na and K currents.

The observation that both TPTA and TPTA MeI (but not TPClφA) seem to “inactivate” the late steady-state current at large depolarization steps was an unexpected result. That is, the late current turns on and rises to a peak, and then decays to a new, lower steady state level. This effect is best seen in the slow sweep, long step duration curves (lower row) of Fig. 3. The time to the peak and the magnitude of the decay of the late current both vary with the membrane potential (Fig. 4). The peak occurs earlier in the step, and the magnitude of the decay (i.e., the difference between the “peak” and “19 msec” I_K curves of Fig. 4) increases, as the depolarization is increased. The
"before" and "after" current-time curves showed only barely measurable decreases in the late current even at very large depolarization steps, after 20–50 msec.

Current-voltage relations for an axon treated with TPTA MeI are shown in Fig. 4. These data are from another "node" from the same axon as the data in Fig. 4. Both the peak initial and late currents are reversibly decreased by TPTA MeI. Similar effects are obtained with TPTA (see Fig. 3), while TPCl has no effect on the current-voltage curves.

* The so-called node is the region of active membrane between two regions of flowing sucrose. If one such node deteriorates, the axon may be pulled through the chamber until a new active region is found.
3. Action of Tropine Esters on Membrane Conductances

Sodium conductance vs. membrane potential curves before and during treatment with TPTA MeI are plotted in Fig. 6. This agent, like TPTA (Goldman and Blaustein, 1966), reduces the maximum sodium conductance, and shifts
**Table 1**

EFFECT OF TROPINE ESTERS ON AXON MEMBRANE CONDUCTANCES

<table>
<thead>
<tr>
<th>Axon</th>
<th>Node</th>
<th>Before (B)</th>
<th>During drug (D)</th>
<th>After (A)</th>
<th>Drug ratio (D/B)**</th>
<th>Activity ratio (A/B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\xi_{Na}$</td>
<td>$\xi_{K}$</td>
<td>$\xi_{leak}$</td>
<td>$\xi_{Na}$</td>
<td>$\xi_{K}$</td>
</tr>
<tr>
<td>L-22-65</td>
<td>1</td>
<td>101</td>
<td>74</td>
<td>15</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>175</td>
<td>75</td>
<td>12</td>
<td>65</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>159</td>
<td>79</td>
<td>10</td>
<td>87</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>3'</td>
<td>131</td>
<td>82</td>
<td>8</td>
<td>59</td>
<td>39</td>
</tr>
<tr>
<td>L-23-65</td>
<td>1'</td>
<td>220</td>
<td>95</td>
<td>22</td>
<td>108</td>
<td>50</td>
</tr>
<tr>
<td>L-24-65</td>
<td>1'</td>
<td>92</td>
<td>36</td>
<td>3</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>L-25-65</td>
<td>1</td>
<td>192</td>
<td>74</td>
<td>23</td>
<td>57</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>176</td>
<td>75</td>
<td>6</td>
<td>86</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>2*</td>
<td>180*</td>
<td>71</td>
<td>2</td>
<td>87</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.43</td>
<td>0.93</td>
<td></td>
<td>0.44</td>
<td>0.86</td>
</tr>
</tbody>
</table>

**Table Note:**

- **Tropine p-tolylacetate**: mMhos/cm²
- **Tropine p-tolylacetate methiodide**: mMhos/cm²

Published March 1, 1968
M. P. Blaisdell
Action of Tropine Esters on Nerve

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (mm)</th>
<th>32</th>
<th>29</th>
<th>220</th>
<th>95</th>
<th>22</th>
<th>0.99</th>
<th>1.02</th>
<th>0.97</th>
<th>1.03</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-23-65</td>
<td>1</td>
<td>215</td>
<td>92</td>
<td>32</td>
<td>212</td>
<td>89</td>
<td>220</td>
<td>95</td>
<td>22</td>
<td>0.99</td>
</tr>
<tr>
<td>L-24-65</td>
<td>1</td>
<td>125</td>
<td>55</td>
<td>4</td>
<td>119</td>
<td>39</td>
<td>8</td>
<td>2</td>
<td>0.95</td>
<td>0.71</td>
</tr>
<tr>
<td>L-25-65</td>
<td>2'</td>
<td>176</td>
<td>75</td>
<td>6</td>
<td>180</td>
<td>71</td>
<td>2</td>
<td>1.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2'</td>
<td>183</td>
<td>67</td>
<td>6</td>
<td>173</td>
<td>68</td>
<td>5</td>
<td>(1.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1'</td>
<td>179</td>
<td>77</td>
<td>11</td>
<td>182</td>
<td>73</td>
<td>6</td>
<td>177</td>
<td>79</td>
<td>12</td>
</tr>
</tbody>
</table>

Average: 1.01 1.00 0.91 1.03

† Drug concentration, 2.5 mm used in all experiments except L-27-65, node 1, in which 0.6 mm was used. Temperatures ranged from 4° to 7°C. The data from L-27-65, node 1, have therefore not been included in the D/B averages.

§ Maximum sodium conductance, calculated as \( g_{Na} = \frac{I_{P1}}{V_{M} - V_{Na}} \), where \( I_{P1} \) is the corrected peak initial current; \( V_{M} \) is the membrane potential; and \( V_{Na} \) is the apparent sodium equilibrium potential; i.e., the positive potential at which \( I_{P1} = 0 \).

∥ Maximum slope potassium conductance, calculated as \( g_{K} = \frac{\Delta I_{K}}{\Delta V_{M}} \), where \( I_{K} \) is the corrected steady-state current (or, in the case of TPTA and TPTA MeI, the maximum late current).

¶ Node refers to the region of active membrane between two regions of flowing sucrose. Occasionally several such regions could be used on a single axon. Node referred to by a prime or double prime means that the same node was tested with several alternating applications of seawater and seawater containing a tropine ester.

** When the first voltage clamp run was made in the presence of drug, and no before data were obtained, during/after ratios were calculated, and are shown in parentheses.

†† In a few instances TPCl16A was used in the washout solution following treatment with TPTA or TPTA MeI. These conductances are referred to by a plus sign (+).
the normalized (dashed line) curve to the right (i.e., in the direction of depolarization) along the voltage axis. Both TPTA (Goldman and Blaustein, 1966) and its quaternary analogue also affect the potassium conductance in similar fashion. TPCIqA has no effect on either the sodium or potassium conductance.

The effects of these three tropine esters on the maximum sodium and potassium conductances are summarized in Table I.

![Graph showing the effects of TPTA MeI on time-to-peak of early transient current](image)

Figure 7. Axon L-26-65. T = 7°C. Time-to-peak of early transient current vs. membrane potential before, during, and after 2.5 mM TPTA MeI.

4. Effect of Tropine Esters on the Rate of Sodium Conductance Turn-On

Fig. 7 shows that TPTA MeI, like TPTA (Goldman and Blaustein, 1966), increases the time from the start of the step depolarization to the peak of the early transient at a given membrane potential. We take the time required to reach the peak of the transient as a measure of the rate of sodium conductance turn-on. The data thus suggest that TPTA and TPTA MeI slow the rate at which the sodium conductance is turned on. TPCIqA did not affect the time-to-peak of the early transient.

DISCUSSION

TPTA and its quaternary analogue, TPTA MeI, reduce both the early transient and late currents and, consequently, the peak sodium and potassium conductances in the voltage-clamped axon. These effects probably account...
for the ability of the two drugs to decrease the amplitude of the action potential in the lobster axon. Both agents also block the action potential in a single node of Ranvier from frog sciatic nerve (Thron et al., 1963; Friess et al., 1966 b). In contrast, TPCI~bA does not block the action potential in the frog node (Thron et al., 1963) or the lobster axon, and does not effect the sodium or potassium currents in the voltage-clamped lobster axon.

Since local anesthetics usually require a hydrophilic amino group for activity (see L6fgren, 1948), the fact that TPTA and TPTA MeI are approximately equally effective in blocking the sodium and potassium conductances in the lobster axon provides strong evidence that the cationic forms of local anesthetics are active. While this does not rule out the possibility that unionized molecules may also be active, the data of Ritchie and Greengard (see their 1966 review) and Dettbarn (1962) support the view that they are inactive.

A second requirement for activity is a lipophilic group (L6fgren, 1948), so that lipid-soluble quaternary nitrogen compounds may have local anesthetic activity (Rosenberg and Ehrenpreis, 1961). Since TPTA and TPTA MeI are both much more soluble in low dielectric constant media than TPCI~bA (Blaustein, 1967), this too, is likely to contribute to the differences in nerve-blocking activity observed here.

The effects of TPTA and TPTA MeI on the voltage-clamped axon have been shown to be qualitatively similar to the effects of procaine in most respects. All three agents reduce the sodium and potassium conductances, slow the rate of sodium conductance turn-on, and shift the normalized sodium conductance vs. voltage curve in the direction of depolarization along the voltage axis (Shanes et al., 1959; Taylor, 1959; Goldman and Blaustein, 1966; Blaustein and Goldman, 1966 b). It, therefore, seems reasonable to assume that procaine and these tropine esters may have a similar mechanism of action on the axon membrane.

The hypothesis for the mechanism of action of barbiturates discussed in the previous paper (Blaustein, 1968; and see Blaustein and Goldman, 1966 a) is also applicable to these data from the cationic anesthetics. Thus, we would expect the lipophilic groups on the drug molecules to dissolve in the membrane lipid, thereby decreasing the membrane sodium and potassium conductance constants. Unlike the barbiturates, which are anions, procaine and the tropines have a positively charged amino nitrogen. This would tend to decrease the net negative charge on the membrane surface, and thereby decrease the amount of calcium bound (i.e., acting as counterions). However, the hydrophilic amino groups from the drug molecules in the membrane will also be acting as counterions, so that the total number of cations available to act as counterions will have increased. Thus, as far as the membrane surface charge is concerned, the effect of introducing a cationic drug could be in-
distinguishable from an increase in the divalent cation concentration. This effect will be manifested by shifts along the voltage axis (in the direction of depolarization) of the ionic conductances and the time- and voltage-dependent conductance parameters, and by prolongation of the action potential.

According to the data and references cited above, it seems reasonable to expect that van der Waals forces between the drug lipophilic and fatty acid chains of membrane lipids, and electrostatic forces between membrane polar groups and the positively charged amino nitrogen are both necessary to coax the cationic drug molecules into the membrane. Interactions involving the membrane "fixed" negative charges may then help to explain the observed antagonism of procaine action by divalent cations (see Goldman and Blaustein, 1966, for references). With a constant cationic drug concentration, as the divalent cation concentration is increased, the number of drug amino groups acting as counterions will decrease. The drug molecules no longer used as counterions will then be released from the membrane as a consequence of the reduction in attractive forces which held them there.

Although the chemical nature of the negatively charged groups in the membrane is unknown, it has been postulated that the polar heads of phospholipids, known to be present in nerve membranes, may provide the negative surface charges. It is therefore of interest that drug and divalent cation interactions with certain phospholipids parallel the properties of the fixed negative charge groups discussed here (see Blaustein and Goldman, 1966 a, for a review and references).

An unusual effect of TPTA and TPTA MeI, and one for which we have no satisfactory explanation, is their action on the late, so-called steady-state current. The shapes of the current-time curves suggest that in the presence of these drugs, after the potassium conductance has been turned on for a short time, it may be partly inactivated. This effect of the tropine esters might possibly be related to the slow inactivation of potassium conductance which has been reported to occur in squid (Ehrenstein and Gilbert, 1966).

The author is indebted to Dr. S. L. Friess for many helpful discussions, and for generously supplying the tropine esters employed in this study; and to Dr. D. E. Goldman for advice and encouragement throughout the course of these experiments.

Received for publication 12 October 1966.

REFERENCES


Blaustein, M. P. 1967. Phospholipids as ion exchangers: implications for a possible


