ABSTRACT
Tetrodotoxin (TTX) at a concentration of $1.6 \times 10^{-7}$M blocked intracellularly recorded action potentials in 12- and 18-day-old embryonic chick atria. However, TTX, $1 \times 10^{-5}$M, did not abolish action potentials recorded from cells in 6-day-old atria. TTX-sensitive receptor sites were present in 6-day-old atrial membranes because the toxin depressed the early Na$^+$ conductance that generated the rapid depolarization phase and the maximum rate of rise of the action potential. TTX at $0.9 \times 10^{-6}$M reduced the maximum rate of rise by 50% in 6-day-old atria, and TTX at $2 \times 10^{-6}$M had the same effect in 12- and 18-day-old preparations. Action potential overshoot changed about 10-20 mV/tenfold variation in external Na$^+$ concentration in 6-day-old atria in contrast to the change of about 60 mV/decade observed in 18-day-old atria. Moreover, the TTX-resistant action potentials in 6-day-old atria depended primarily on an inward Ca$^{2+}$ current because overshoot changed 24 mV/decade variation in external Ca$^{2+}$ concentration. The improvement in sodium-electrode properties that occurred during maturation was accompanied by an increase in the maximum rate of rise of the action potential, which averaged 64 v/sec in 6-day-old atria and 94 v/sec in 18-day-old atria. These findings suggested that the early Na$^+$ conductance mechanism became more critical in generating the action potential in older embryonic atria. These data were consistent with the hypothesis that the membrane density of early conductance sites increased with embryonic age. The TTX resistance of 6-day-old atria depended on an inward Ca$^{2+}$ current that generated action potentials after the early Na$^+$ conductance sites had been blocked by the toxin.

KEY WORDS membrane potential sodium conductance amplitude sodium electrode maximum rate of rise tetrodotoxin receptor overshoot calcium conductance embryogenesis calcium electrode

I recently proposed (1) that changes in membrane conductance properties, rather than changes in the cholinergic receptor, were primarily responsible for the alterations in the membrane actions of acetylcholine (ACh) observed in embryonic pacemaker cells. ACh depolarized noninnervated embryonic chick pacemaker cells by increasing membrane conductance to Na$^+$; in the absence of Na$^+$, ACh hyperpolarized the membrane because it also increased membrane conductance to K$^+$. In the innervated preparation, ACh hyperpolarized the cells, and the ability of the drug to augment K$^+$ conductance increased during maturation. Thus, the reduction in atrial action potential duration by ACh, an effect also due to an increase in K$^+$ conductance, became greater as development proceeded. Although the augmentation of K$^+$ conductance by ACh and by elevated external K$^+$ concentration increased during maturation, the improvement in potassium-electrode properties of atrial membranes was not reflected in a substantial change in membrane potential between the sixth and the eighteenth day of development. I considered the possibility that a change in membrane ionic conductance produced by other drugs might also occur during develop-
ment. Tetrodotoxin (TTX) was selected for this study because it blocked the early transient conductance (normally Na⁺ conductance) in excitable cells (2-4). The close relationship between the TTX receptor and the early conductance site (4) required that I also examine the effects of Na⁺ on atrial action potentials. In mature cardiac fibers, the maximum rate of rise of the action potential depended on the external Na⁺ concentration ([Na⁺]) (5-7). Voltage clamp measurements also showed that an early inward Na⁺ current generated the maximum rate of rise of the action potential (8-10). Moreover, TTX depressed the maximum rate of rise and the early inward Na⁺ current in mature amphibian and mammalian cardiac muscle (8-11).

Therefore, experiments were conducted to study the effects of TTX and [Na⁺], on the maximum rate of rise and the amplitude of atrial action potentials at selected times during development.

Methods

Fertilized White Leghorn eggs were maintained at 37.5°C in an incubator until the time for experiments. The heart was removed from the embryo and pinned to the silicone rubber floor of a tissue chamber (10 ml in volume) containing Tyrode's solution of the following millimolar composition: K⁺ 2.7, Na⁺ 149, Ca²⁺ 1.8, Mg²⁺ 1.0, Cl⁻ 145, HCO₃⁻ 11.9, H₂PO₄⁻ 0.4, and glucose 5.5. The ventricles were removed except in the younger hearts, and an opening was made in the atria according to methods described by others (12) to facilitate impalements of atrial cells from the endocardial surface. After isolation, all preparations were allowed to equilibrate for 30-45 minutes in Tyrode's solution that flowed at a rate of 2 ml/min. Aeration of the bath with a mixture of 95% O₂-5% CO₂ resulted in a pH of 7.3, and bath temperature was maintained at 30 ± 1°C for all experiments. Rhythmic spontaneous electrical activity was maintained for 6-8 hours under these conditions.

In some experiments, the preparations were exposed to various concentrations of Na⁺ in the bathing fluid. Saline solutions with the desired [Na⁺], were prepared by mixing appropriate volumes of the standard Tyrode's solution and an isotonic sucrose stock solution having the following millimolar composition: K⁺ 2.7, Na⁺ 12, Ca²⁺ 1.8, Mg²⁺ 1.0, Cl⁻ 8, HCO₃⁻ 11.9, H₂PO₄⁻ 0.4, sucrose 240, and glucose 5.5. Appropriate volumes of standard Tyrode's solution and calcium-free Tyrode's solution were mixed to obtain reduced concentrations of Ca²⁺ ([Ca²⁺]), for selected experiments. No osmotic adjustment was made when [Ca²⁺] was increased by addition of CaCl₂. An equilibration period of 10-15 minutes elapsed before membrane potentials were recorded in each solution. Cation concentrations (Na⁺, K⁺, Ca²⁺, and Mg²⁺) in the bath fluid were analyzed routinely. The Na⁺ and K⁺ measurements were made with a modified flame photometer with filters (Baird Atomic) that minimized interference of Na⁺ with K⁺, and the Ca²⁺ and Mg²⁺ analyses were performed with an atomic absorption spectrophotometer (Perkin Elmer 290B).

Impalements were made with glass capillary microelectrodes filled with 3M KCl (tip resistance 20-35 Mohms). The microelectrodes had tip potentials of 5 mv or less, and stable recordings of membrane potentials were obtained in all solutions, even when the ionic strength was reduced by replacement of NaCl with sucrose. The reliability of potential measurements in sucrose-containing solutions was verified by the fact that the resting potentials did not vary from values obtained in sucrose-free control saline by more than ±3 mv. A chloridized silver wire connected the microelectrode to a high input impedance preamplifier having capacity neutralization that was adjusted for optimal recording of potential transients in all portions of the experiments; the indifferent bath electrode was a chloridized silver wire. Signals were led from the preamplifier to one channel of a dual-beam oscilloscope. The maximum rate of rise of the action potential was obtained by a passive differentiator circuit (time constant 10⁻⁴ seconds) and displayed on the second channel of the oscilloscope. Calibration of the recording system with sinusoidal wave forms showed that the response of the differentiating circuit was linear over the range of 10 to 200 v/sec. Velocities less than 10 v/sec were measured directly from photographic enlargements and were accurate to within ±10%. The preparations were often stimulated by supramaximal rectangular pulses (0.5 msec, 4-10 v) applied to the atria through platinum electrodes at a frequency of 0.5 Hz.

The flow of saline solution was temporarily stopped when TTX was added to the bath. Arresting the flow of solution by itself had no effect on the electrical activity of the preparations. Measurements are given as the mean ± se.

Results

MEMBRANE POTENTIALS IN EMBRYONIC CHICK ATRIA

Values of the resting potential and the

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amplitude and the maximum rate of rise of the action potential in 6-, 12-, and 18-day-old embryonic chick atria are shown in Table 1. These data are a summation of the measurements obtained in the control periods of experiments in which TTX was added or $[\text{Na}^+]_o$ was changed. The resting potential did not vary appreciably with embryonic age, and the mean value in 12-day-old atria was not significantly greater ($P = 0.5$) than those observed in 6- and 18-day-old atria. Action potential amplitude (the maximum amplitude of the entire action potential) was also unchanged during development. However, a significant increment in the maximum rate of rise of the action potential occurred at each stage examined.

TABLE 1

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>$E_m$ (mV)</th>
<th>Amplitude (mV)</th>
<th>$V_{\text{max}}$ (V/sec)</th>
<th>$N$</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-66 ± 1</td>
<td>89 ± 1</td>
<td>64 ± 3</td>
<td>60</td>
</tr>
<tr>
<td>12</td>
<td>-69 ± 1</td>
<td>90 ± 1</td>
<td>78 ± 2*</td>
<td>63</td>
</tr>
<tr>
<td>18</td>
<td>-67 ± 1</td>
<td>91 ± 1</td>
<td>94 ± 3*</td>
<td>55</td>
</tr>
</tbody>
</table>

$E_m =$ resting membrane potential, $V_{\text{max}} =$ maximum rate of rise of the action potential, $N =$ number of observations.

* $P < 0.01$ when compared to value in younger heart.

TTX blockaded the action potentials in 12- and 18-day-old atria were similar (Fig. 1). Action potentials and their maximum rate of rise were recorded from a single cell in an electrically driven $(10 \text{ V}, 0.5 \text{ msec})$ preparation from an 18-day-old heart (Fig. 1A–C). TTX $(5 \times 10^{-6} \text{M})$ reduced the amplitude by $20\%$ within 1.5 minutes, and the maximum rate of rise was diminished (Fig. 1B). Excitation block occurred within 5 minutes (Fig. 1C), and intense stimuli $(150 \text{ V}, 10 \text{ msec})$ did not elicit a propagated action potential. TTX produced comparable effects on spontaneously occurring action potentials from a 12-day-old preparation (Fig. 1D–F). Both the amplitude and the maximum rate of rise declined within...
TTX-resistant action potentials in a 6-day-old atrial cell. Calibrations are given as in Figure 1. Horizontal lines in A and D are zero potential levels. All records are taken from a single impalement. A: Control. B: 10 minutes after addition of TTX to a concentration of 2 × 10⁻⁷ M. C: 7 minutes after 5 × 10⁻⁶ M TTX was added. D: 5 minutes after 2 × 10⁻⁶ M TTX was added. E: 10 minutes after 7 × 10⁻⁵ M TTX was added. F: 10 minutes after 1 × 10⁻⁴ M TTX was added.

1 minute after addition of 6 × 10⁻⁸ M TTX (Fig. 1E), and spontaneously generated action potentials were blocked in 4 minutes (Fig. 1F). In addition, action potentials could not be evoked by external stimuli. The effects of TTX were reversible, and complete recovery of both maximum rate of rise and amplitude required about 1 hour after exposure to TTX for about 10 minutes.

TTX did not block action potentials in 6-day-old atria, but it depressed their maximum rate of rise, as shown in Figure 2. A slight reduction in amplitude accompanied the decline in the maximum rate of rise caused by 2 × 10⁻⁸ M TTX (Fig. 2B). The deflection indicating the maximum rate of rise of the action potential was reduced to a negligibly small value in the presence of 5 × 10⁻⁷ M TTX (Fig. 2C). The differentiator time constant was too brief to record the slow velocity, and the velocity was measured from photographic enlargements at expanded sweep. The membrane potential declined by about 5 mV, but action potentials persisted at about 90% of the control amplitude. Additional increments in the TTX concentration (2 × 10⁻⁶ to 1 × 10⁻⁶ M), as shown in Figure 2D–F, did not produce any further changes either in the amplitude and the contour of the action potential or in the resting membrane potential. Moreover, the maximum rate of rise remained at 5–10 V/sec, the value in 5 × 10⁻⁸ M TTX.

Concentration-effect curves were obtained for the actions of TTX on 6-, 12-, and 18-day-old atria (three experiments each). The degree of blockade of the maximum rate of rise and the amplitude of the action potential in each age group was obtained by plotting the values of these parameters in the presence of TTX as a fraction of those observed in the control period (Fig. 3). There were no appreciable differences in the sensitivity of the maximum rate of rise to TTX in 12- and 18-day-old atria (50% reduction in maximum rate of rise with 2 × 10⁻⁶ M TTX). The data from 6-day-old atria suggest that the maximum rate of rise (50% reduction at 0.9 × 10⁻⁸ M TTX) was somewhat more sensitive to TTX than it was in older hearts. Action potential amplitude in 12- and 18-day-old atria declined only after the maximum rate of rise had been reduced to 60% of control in 1.6 × 10⁻⁷ M TTX. A precipitous reduction in amplitude occurred between TTX concentrations of 3.1 × 10⁻⁸ and 1.6 × 10⁻⁷ M, and action potentials were completely blocked at the latter concentration. By contrast, action potential amplitude was di-
TETRODOTOXIN AND EMBRYONIC ATRIA

Graphic summation of TTX action on the amplitude (amp) and the maximum rate of rise (Vmax) of action potentials in 6-, 12-, and 18-day-old embryonic chick atria. Each symbol is the mean value of 15–30 impalements.

Effects of External Sodium Concentration on Atrial Action Potentials

Reductions in [Na+]o had no effect on the resting membrane potential but reversibly diminished both overshoot and the maximum rate of rise. Overshoot did not change when [Na+]o ranged from 100 to 150 mM in 18-day-old atria (Fig. 4A), but it declined sharply when [Na+]o was less than 100 mM. The relationship between overshoot and [Na+]o (slope of about 60 mV/tenfold change in [Na+]o) indicated that the membrane displayed sodium-electrode properties at the peak of the action potential in 18-day-old atria. The preparations became inexcitable when [Na+]o was less than 30 mM, and automaticity failed when [Na+]o was less than 50–55 mM. The effects of [Na+]o on overshoot in 12-day-old atria (slope of 50 mV/decade) were similar to those in 18-day-old atria. The results obtained in 6-day-old atria (Fig. 4B) resembled those in 18-day-old atria in two respects. (1) Overshoot did not change when [Na+]o ranged from 100 to 150 mM.
Excitability failed when \([Na^+]_o\) was less than 30 mM. However, the slope of the plot of overshoot vs. \([Na^+]_o\) (10-20 mv/decade) deviated considerably from the theoretical limit for a sodium electrode (60 mv/decade). Automaticity also failed when \([Na^+]_o\) was less than 30 mM. It is noteworthy that action potentials in 6-day-old atria depended to a large extent on \([Ca^{2+}]_o\) (three experiments). In the presence of TTX \((3.1 \times 10^{-8} \text{M})\), reductions in \([Ca^{2+}]_o\) reversibly diminished overshoot without changing the resting potential (Fig. 5). Regression analysis showed that overshoot changed 24 mv/tenfold change in \([Ca^{2+}]_o\) when \([Ca^{2+}]_o\) ranged from 0.50 to 1.7 mM. Action potentials were abolished when \([Ca^{2+}]_o\) was less than 0.50 mM. The fact that overshoot at \([Ca^{2+}]_o\) of 3.6 mM deviated from the relationship obtained at lower \([Ca^{2+}]_o\) could reflect the membrane-stabilizing action of the ion on its own conductance mechanism. The combined effects of TTX and Na+ deficiency were also examined in 6-day-old atria (two experiments). As noted previously, action potential amplitude declined about 10% when atria were bathed in saline solutions in which either TTX \((3.1 \times 10^{-8} \text{M})\) had been added or \([Na^+]_o\) had been reduced to 50%. However, reduction of \([Na^+]_o\) to 50% (72 and 80 mM) in the presence of TTX \((3.1 \times 10^{-8} \text{M})\) abolished spontaneous and evoked action potentials.

Because of the difference in the absolute magnitude of the maximum rate of rise in 6-, 12-, and 18-day-old atria, the relative magnitude of this parameter was plotted to compare the results produced by changes in \([Na^+]_o\). As shown in Figure 6, the effects of \([Na^+]_o\) on the maximum rate of rise were practically identical in 6-, 12-, and 18-day-old atria, in contrast to the findings for overshoot. The dependence of the maximum rate of rise on \([Na^+]_o\) was more prominent at lower \([Na^+]_o\). A reduction in \([Na^+]_o\) from 100 to 60% (150 to 90 mM) reversibly diminished the maximum rate of rise of the action potential by about 20%. However, when \([Na^+]_o\) ranged from 60 to 20% (90 to 30 mM), the maximum rate of rise declined by almost 60%. Extrapolation of the curve through a maximum rate of rise of zero suggested that action potentials would be negligibly small when \([Na^+]_o\) was less than 30 mM, in agreement with the effects of \([Na^+]_o\) on excitability.
TETRODOTOXIN AND EMBRYONIC ATRIA

Discussion

The maximum rate of rise of atrial action potentials increased significantly during embryonic development in the chick, but the amplitude did not change. Concomitantly, atrial action potentials underwent a transformation in their sodium-electrode properties and in their susceptibility to blockade by TTX. Action potentials in 6-day-old atria persisted in the presence of TTX concentrations that were 20–100 times greater than those sufficient to abolish action potentials in 12- and 18-day-old atria. These changes pointed to a developmental transition in the membrane ionic conductance initiating the atrial action potential.

The early conductance mechanism in embryonic atrial cells of all ages depended on Na⁺ because the maximum rate of rise varied in direct proportion to [Na⁺]o. That the maximum rate of rise signaled the operation of an early Na⁺ conductance system in embryonic atria agreed with the conclusions of others who studied action potential generation in mature vertebrate hearts (5-10) and in embryonic chick ventricular cells (13). In the latter preparation, neither the maximum rate of rise nor its dependence on Na⁺ changed during development. Moreover, the slope of the plot of overshoot vs. log [Na⁺]o (61 mv/decade) did not vary, and it was concluded that the specific increase in Na⁺ conductance that generated the ventricular action potential was established by the sixth day and did not change through the nineteenth day of development. Although the effects of [Na⁺]o on the relative value of the maximum rate of rise were the same in all atrial groups, the maximum rate of rise increased by almost 50% between the sixth and eighteenth day. These findings suggested that the contribution of the early Na⁺ conductance system to generation of atrial action potentials increased during maturation.

In 6-day-old atria, overshoot declined only 10–20 mv for a tenfold reduction in [Na⁺]o when [Na⁺]o ranged from 30 to 100 mM. However, the overshoot of 18-day-old action potentials fell 60 mv for a tenfold diminution in [Na⁺]o, an indication that sodium-electrode properties had improved considerably. The fact that an improvement in sodium-electrode properties during overshoot accompanied an increase in the maximum rate of rise during embryogenesis gave additional support to the hypothesis that participation of the early Na⁺ conductance system in action potential generation had increased. Measurements of internal Na⁺ concentration ([Na⁺]i) in embryonic ventricles gave estimates of 80 mM and 30 mM, respectively, at 6 days and at hatching (14). Assuming that a similar change occurred in atrial [Na⁺]i, it would be anticipated that the driving force for Na⁺ entry increased between 6 and 18 days. Nevertheless, reductions in [Na⁺]o (to as low as 30 mM) would be expected to reduce overshoot by 60 mv/decade only if the ratio of Na⁺ conductance to K⁺ conductance at the peak of the action potential was equal in 6- and 18-day-old atria. The fact that overshoot did not change when [Na⁺]o was greater than 100 mM in 6- and 18-day-old atria has also been noted in uterine smooth muscle (15) and in cultured embryonic ventricular cells (16, 17). This phenomenon could result from saturation of the sodium-carrier system at [Na⁺]o greater than 100 mM.

Depression of the early Na⁺ conductance mechanism in embryonic atria by TTX, like that caused by reduced [Na⁺]o, did not change, as judged by the concentration-effect relationships for the actions of TTX on the fractional maximum rate of rise. The cause of the unexpected depolarization (4–5 mv) associated with TTX action in 6-day-old atria is not known. However, it is not likely that the small depolarization significantly modified the TTX-maximum rate of rise relationship. The TTX concentration that reduced the maximum rate of rise by 50% ranged from 0.9 × 10⁻⁸ to 2 × 10⁻⁸M in 6- and 18-day-old atria (as estimated from interpolation of the concentration-effect relationships), and these data showed that pharmacologically similar receptor sites were present. Embryonic atrial sensitivity to TTX can be compared with that in some mature cardiac tissues. For example,
2.5 x 10^{-8} \text{ g TTX/ml} \ (8 \times 10^{-8} \text{M}) \ reduced \ the \ maximum \ rate \ of \ rise \ of \ frog \ ventricular \ action \ potentials \ by \ 83\% \ (11), \ and \ overshoot \ declined \ after \ exposure \ to \ TTX \ for \ more \ than \ 30 \ minutes. \ Conduction \ block \ supervened \ at \ a \ concentration \ of \ 5 \times 10^{-8} \ \text{g/ml} \ (1.6 \times 10^{-7} \text{M}), \ just \ as \ it \ did \ in \ 12- \text{and} \ 18-day-old \ atria. \ Similarly, \ 1 \times 10^{-7} \text{M} \ TTX \ abolished \ the \ early \ inward \ Na^{+} \ current \ and \ the \ rapid \ depolarization \ phase \ of \ the \ frog \ atrial \ action \ potential \ (8, \ 10). \ The \ concentration \ of \ TTX \ that \ reduced \ the \ maximum \ rate \ of \ rise \ by \ 50\% \ was \ only \ five- \text{to} \ tenfold \ greater \ than \ the \ concentrations \ of \ TTX \ and \ saxitoxin \ needed \ to \ depress \ Na^{+} \ conductance \ by \ 50\% \ in \ the \ squid \ giant \ axon \ (3.3 \times 10^{-9} \text{M} \ [18]) \ and \ in \ the \ frog \ node \ of \ Ranvier \ (1.2 \times 10^{-9} \text{M} \ [4]).

TTX-resistant action potentials in 6-day-old atria depended primarily on an inward Ca^{2+} current. The slope of the plot of overshoot vs. \text{[Ca^{2+}]}, \ (24 \text{ mv/decade}), \ although \ slightly \ less \ than \ the \ theoretical \ limit \ for \ a \ perfect \ calcium \ electrode \ (30 \text{ mv/decade}), \ is \ greater \ than \ that \ observed \ in \ the \ frog \ ventricle \ (18 \text{ mv/decade}) \ during \ the \ calcium-dependent \ phase \ of \ the \ action \ potential \ (19). \ A \ quantitative, \ as \ well \ as \ a \ qualitative, \ change \ in \ conductance \ properties \ also \ occurred \ because \ the \ sodium-electrode behavior \ of \ 18-day-old \ atria \ improved \ over \ that \ in \ 6-day-old \ atria. \ Perhaps \ the \ slow \ TTX-resistant \ Ca^{2+} \ conductance \ system \ was \ also \ modified \ during \ development. \ Action \ potentials \ insensitive \ to \ TTX \ have \ been \ observed \ in \ cultured \ chick \ ventricular \ cells \ (17, \ 20), \ which \ share \ some \ similarity \ with \ embryonic \ atria. \ For \ example, \ the \ maximum \ rate \ of \ rise \ ranged \ from \ 5 \ to \ 10 \ \text{v/sec} \ in \ cultured \ cells \ in \ the \ absence \ and \ the \ presence \ of \ TTX \ and \ in \ embryonic \ atrial \ cells \ in \ the \ presence \ of \ TTX. \ However, \ cultured \ cells \ displayed \ a \ TTX \ receptor \ only \ for \ resting \ Na^{+} \ conductance \ (21) \ and \ not \ for \ the \ Na^{+} \ conductance \ system \ generating \ the \ action \ potential. \ The \ intriguing \ aspect \ of \ embryonic \ atrial \ cells \ lies \ in \ the \ in vivo \ transformation \ of \ membrane \ properties \ that \ rendered \ the \ action \ potential \ sensitive \ to \ TTX. \ It \ is \ interesting \ to \ note \ that \ TTX \ (3 \times 10^{-5} \text{M}) \ reduced \ the \ maximum \ rate \ of \ rise \ to \ the \ same \ extent \ in \ acutely \ and \ chronically \ denervated \ skeletal \ muscle \ (22). \ However, \ TTX \ (10^{-5} \text{M}) \ blocked \ excitation \ in \ acutely \ denervated \ muscles, \ but \ concentrations \ up \ to \ 10^{-5} \text{M} \ did \ not \ abolish \ action \ potentials \ in \ chronically \ denervated \ preparations. \ Moreover, \ the \ maximum \ rate \ of \ rise \ declined \ by \ 33\% \ (from \ 600 \ to \ 400 \ \text{v/sec}) \ in \ chronically \ denervated \ muscle. \ Temporal \ development \ of \ resistance \ to \ TTX \ paralleled \ the \ spread \ of \ chemosensitivity \ to \ applied \ ACh, \ although \ the \ receptor \ sites \ for \ TTX \ and \ ACh \ remained \ pharmacologically \ distinct \ (23). \ Vagal \ innervation \ of \ embryonic \ chick \ atria, \ which \ begins \ on \ the \ fifth \ incubation \ day \ (24), \ could \ become \ extensive \ during \ maturation. \ However, \ the \ possibility \ that \ vagal \ innervation \ somehow \ increased \ atrial \ responsiveness \ to \ ACh \ appeared \ remote. \ Action \ potentials \ from \ the \ ventricle, \ which \ is \ not \ innervated \ by \ the \ vagus, \ also \ underwent \ a \ transition \ from \ TTX-resistant \ to \ TTX-sensitive \ status \ between \ the \ third \ and \ sixth \ incubation \ day, \ i.e., \ earlier \ than \ in \ the \ atrium \ (25). \ The \ appearance \ of \ TTX-resistant \ action \ potentials \ and \ spread \ of \ ACh \ sensitivity \ have \ been \ attributed \ to \ genetically \ induced \ protein \ synthesis \ because \ cycloheximide \ and \ actinomycin \ D \ prevented \ these \ effects \ of \ denervation \ (26).

The actions of TTX on embryonic chick ventricle have been reported (25, 27). High concentrations of TTX (6 \times 10^{-5} \text{M}) had no effect on electrical activity in hearts younger than 5 days and blocked action potentials in hearts older than 7 days (25). Moreover, the depressant actions of TTX occurred only when the control values of the maximum rate of rise were high. The lack of TTX action was attributed to inactivation of early Na^{+} conductance by lower resting potentials or to a lack of receptors (i.e., insensitivity), as was also suggested for embryonic rat ventricular muscle (28). Although there is general agreement between these studies and my own, the actions of TTX on embryonic chick atria were not caused by either lower resting potential or a lack of TTX receptors. The significance of the present study resided in two important relationships. First, membrane receptor sensi-
TETRODOTOXIN AND EMBRYONIC ATRIA

Activity to TTX did not change, although the susceptibility of action potentials to blockade by TTX increased with maturation. Second, the fractional dependence of Na+ conductance on [Na+]o did not vary, although the sodium-electrode properties of the overshoot improved during development. If one assumes an intimate relationship between the Na+ “channels” (an operational definition) and the TTX receptor in embryonic atria, as occurs in other excitable cells (4, 18, 29, 30), it can be speculated that the density, but not the chemical properties and the kinetics, of early Na+ conductance channels responsible for generating the maximum rate of rise increased with developmental age. The interplay of [Na+]o, the maximum rate of rise, and TTX in embryonic atria can be compared to an enzyme-substrate reaction system. The maximum velocity of an enzyme-substrate reaction is directly proportional to the enzyme concentration (31). However, the substrate concentration at which velocity is half-maximal will be the same for different concentrations of the same enzyme, i.e., the K_m values are equal. The analogy of this system to embryonic atria can be seen if one replaced substrate concentration with [Na+]o, reaction velocity with the maximum rate of rise of the action potentials, and enzyme concentration with early Na+ conductance sites. Thus, the age-dependent increase in control values of the maximum rate of rise of the action potential would be referable to an increased concentration or density of Na+ conductance sites. An increased density of early Na+ conductance sites could also account for optimal control of overshoot by [Na+]o in 18-day-old atria. Accordingly, blockade of action potentials by TTX in older hearts resulted from elimination of the increased contribution of the TTX-sensitive Na+ conductance component to action potential generation rather than from a change in receptor sensitivity. In this context, it is helpful to consider a view of Na+ channel operation in the voltage-clamped node of Ranvier (32). It was suggested that axons with a smaller Na+ conductance per unit of membrane area had fewer Na+ channels occupying the node than did axonal preparations with a larger Na+ conductance. Experiments designed to test both the chemical specificity of the early Na+ conductance mechanism and the effects of temperature on the sensitivity of the maximum rate of rise and overshoot to [Na+]o, and TTX will be helpful in testing the validity of the density hypothesis. Clearly, this study has shown that developmental transitions in membrane ionic conductance modulated the pharmacological actions of TTX in embryonic atria, a phenomenon previously noted with ACh (1).

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References
Action Potentials in Chick Atria: INCREASED SUSCEPTIBILITY TO BLOCKADE BY TETRODOTOXIN DURING EMBRYONIC DEVELOPMENT

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