Calcium Current Channels Induced by Catecholamines in Chick Embryonic Hearts Whose Fast Sodium Channels are Blocked by Tetrodotoxin or Elevated Potassium

By Koki Shigenobu and Nick Sperelakis

ABSTRACT

In isolated ventricles of old (9-19 day) chick embryonic hearts made inexcitable by inactivating the fast sodium channels with either tetrodotoxin (TTX) or elevated external potassium, catecholamines induced slow electrical responses and concomitant contractions within 1-3 minutes. These slow responses had a higher threshold and propagated at a slower velocity than did the normal action potential. They were often graded and quite variable from one region of the heart to another. They were blocked by lanthanum or manganese (1 mM) and showed a dependence on the external calcium level. Strontium and barium could substitute for calcium. The slow channels were not completely inactivated when the external potassium concentration was raised until low membrane potentials of -10 to -25 mv were reached. The catecholamine-induced responses had a shape similar to that of the plateau component of the normal action potential, and cooling affected both in a similar manner. In some cases, slow responses persisted in TTX-blocked hearts even without the addition of exogenous catecholamines. Cyclic 3',5' AMP, dibutyryl cyclic AMP, theophylline, and caffeine partially mimicked the catecholamines, but usually the development of the response to these drugs was much slower. Prostaglandins, ryanodine, and acetylcholine had no effect. The order of effectiveness of the catecholamines was isoproterenol > epinephrine ≥ norepinephrine > dopamine ≥ dopa. Phenylalanine and tyrosine were ineffective. Isoproterenol produced a near-maximal effect at 5 X 10⁻⁷ M. Propranolol blocked the catecholamine-induced effects. In young (2-5 day) hearts, there was no evidence that catecholamines produced a similar effect, and they did not have a positive inotropic action or an effect on the slow sodium channels. The results suggest that catecholamines quickly increase the density of divalent cation channels available in the sarcolemma of older chick hearts, thereby increasing the inward Ca²⁺ current and influencing contraction.

KEY WORDS
catecholamine ineffectiveness in young embryonic hearts
excitation-contraction coupling
isoproterenol and epinephrine effects
cardiac action potential plateau
lanthanum blockade of calcium current
cyclic AMP effect on calcium current
ryanodine ineffectiveness
manganese blockade of calcium current

tetrodotoxin (TTX) blocks fast sodium (Na⁺) channels (1) but has no effect on slow cation channels, whether they admit calcium (Ca²⁺) or Na⁺, or both (2). We previously showed that ventricular cells of young (2-4 day) chick hearts do not have fast Na⁺ channels but instead have slow Na⁺ channels which (1) are insensitive to TTX, (2) are inactivated only at lower resting potentials, (3) do not admit lithium (Li⁺), and (4) do not pass Ca²⁺ (3, 4). The slow channels cause a low maximum rate of rise of the action

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potential (+V\text{max}) for the same takeoff potential compared with that in older cells which possess fast Na\textsuperscript{+} channels. That is, the low +V\text{max} at the natural resting potential is not due to partial inactivation of fast Na\textsuperscript{+} channels. By day 8, the slow Na\textsuperscript{+} channels are completely replaced by fast Na\textsuperscript{+} channels. In a transition period (days 5–7), both slow and fast Na\textsuperscript{+} channels coexist, and TTX merely reduces +V\text{max} to a minimum value of about 5–15 v/sec. The fact that +V\text{max} continues to increase during development (until its peak is reached at about day 18) even after the resting potential has about reached its peak level (at about day 8) suggests that the density of fast Na\textsuperscript{+} channels continues to increase during later development. When placed into culture, cells taken from older embryonic hearts revert back to the early embryonic state with respect to the Na\textsuperscript{+} channels: the TTX-sensitive fast channels which do admit Li\textsuperscript{+} are replaced by TTX-insensitive slow channels which do not admit Li\textsuperscript{+} (5–7), and the cells do not respond (electrically) to catecholamines (5). It has been reported that, in cardiac muscles blocked by TTX or elevated external potassium ([K\textsuperscript{+}]\textsubscript{o}), catecholamines restore excitability in the form of slow responses. Such observations were made in frog atrium (8–10), cow Purkinje fibers (11), sheep Purkinje fibers (12), guinea pig papillary muscles (8), and guinea pig atrium (13). Therefore, the present experiments were done to determine whether Ca\textsuperscript{2+} channels could be detected in embryonic chick ventricular cells. We found that catecholamines did indeed induce Ca\textsuperscript{2+} channels in older hearts in which the fast Na\textsuperscript{+} system was inactivated by TTX or elevated [K\textsuperscript{+}]\textsubscript{o}; however, catecholamines had no discernible effect in young hearts.

**Methods**

Embryonic hearts were removed from fertilized chicken eggs (White Leghorn, Babcock strain) at various stages of development. Each heart was pinned by its edges in a chamber. In most experiments, microelectrode penetrations were made into the endocardial surface of the right ventricular wall. The preparations were bathed in modified Ringer’s solution having the following ionic composition (mM): Na\textsuperscript{+} 150, K\textsuperscript{+} 3, Ca\textsuperscript{2+} 2, Mg\textsuperscript{2+} 1, HCO\textsubscript{3}\textsuperscript{-} 15, and Cl\textsuperscript{-} 144. The solution was bubbled with 95% O\textsubscript{2} - 5% CO\textsubscript{2}. The bath temperature was maintained at 37 ± 1°C, except during cooling experiments. The muscles were stimulated by platinum electrodes usually arranged for bipolar stimulation; sometimes field stimulations were applied. The ventricles were about 8 mm in diameter, and most penetrations were made near the middle about 4 mm from the electrodes.

Conventional intracellular recording was done using glass capillary microelectrodes filled with 3M KCl. The microelectrode resistance was usually about 30 megohms. The reversible half-impedance probe and capacitance neutralization was used, and the signal was led to a Tektronix 565 dual-beam oscilloscope. The maximum rate of rise of the action potential (+V\text{max}) or the slow response and dV/dt were measured using a Tektronix type O operational amplifier for electronic differentiation. The dV/dt channel was purposely not aligned with the voltage-time channel so as not to obscure +V\text{max} within the rising phase of the action potential.

In some experiments, the mechanical activity of the heart was recorded simultaneously with the intracellular microelectrode recording. In a few experiments, only the mechanical activity was recorded. A Grass FT-0.03 force transducer was used for recording the contractions (nearly isometric) of the older hearts. For the very small young (3–5 day) hearts, a piezoelectric crystal phonograph cartridge needle (Astatic model 18) was positioned on the surface of the wall of the heart and used for recording movements of the wall (3).

The following drugs were used: tetrodotoxin (Calbiochem Company, Sankyo, crystalline 3X), l-epinephrine bitartrate, l-norepinephrine bitartrate, l-isoproterenol bitartrate, l-phenylalanine, l-tyrosine, l-dopa, l-dopamine, dl-propranolol HCl, cyclic 3',5'-adenosine monophosphate (AMP), dibutryl cyclic AMP, theophylline, caffeine, prostaglandins E\textsubscript{1} and F\textsubscript{2a} (trimethamine salt),\textsuperscript{1} ryanodine,\textsuperscript{2} and acetylcholine HCl. These drugs were dissolved in distilled water in a high concentration, and small amounts of concentrated stock solution were added to give the final bath concentration.

In some experiments, small volumes of concentrated LaCl\textsubscript{3} or MnCl\textsubscript{2} solutions were added to

\textsuperscript{1}Generously supplied by Dr. John E. Pike of the Upjohn Company.
\textsuperscript{2}Generously supplied by Dr. Edward F. Rogers of Merck, Sharpe & Dohme Research Laboratories.
the bath to give a final concentration of 1 mM. In the case of lanthanum (La^{3+}), the Ringer solution was modified (HCO\textsubscript{3}\textsuperscript{-}-free and PO\textsubscript{4}\textsuperscript{3-}-free; Tris-HCl buffer, 5 mM) to prevent precipitation of the La\textsuperscript{3+}. The high-K\textsuperscript{+} solutions were made by substitution of KCl for NaCl in the Ringer solution, keeping the sum of [K\textsuperscript{+}]\textsubscript{o} and [Na\textsuperscript{+}]\textsubscript{o} constant at 153 mM; all other ion concentrations were kept the same. In some cases, [K\textsuperscript{+}]\textsubscript{o} was increased by small amounts by adding a small volume of concentrated KCl solution to the bath. Ca\textsuperscript{2+}-free solutions were made by removing only the CaCl\textsubscript{2} from the Ringer solution. In some experiments, the Ca\textsuperscript{2+} concentration of the bath was lowered or raised by adding a small volume of concentrated ethyleneglycol bis(\textbeta-aminoethyl ether)-N,N\textprime-tetraacetic acid (EGTA) or CaCl\textsubscript{2} solution. The standard

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**FIGURE 1**

Some characteristics of the catecholamine-induced responses in old (10-16 day) chick embryonic ventricular cells from five hearts. First Row: A 14-day heart. A: Normal control response. B: Impalement of another cell showing complete failure of excitability in the presence of TTX (4 \textmu g/ml) even at a stimulus intensity tenfold greater than normal. C: Response after nor-epinephrine (NE, 10^{-4}M), showing dependency on current intensity (rectangular pulses at constant duration of 5 msec). Second Row: A 16-day heart. D: Control. E: Failure in TTX. F: After epinephrine (Epi, 10^{-4}M), stimulus intensity was varied at a constant duration of 5 msec (several sweeps superimposed). G: A 14-day heart. Epinephrine-induced responses in presence of TTX, showing the decrease in magnitude and duration produced by decreases in stimulus duration from 5 to 1 msec (intensity constant). H: A 16-day heart. Several sweeps superimposed at different stimulus intensities (duration of 5 msec) showing double response in a cell. The first response was due to direct stimulation by strong applied current, and the second response was due to propagation at small current. I: Isoproterenol (ISO)-induced spontaneous action potentials (SPONT) in presence of TTX in a 10-day heart. Voltage calibration in B applies to all panels, and time calibration in B applies to all except H and I. Broken horizontal line in all panels gives the zero potential level. Arrow in C points to shock artifact. Field stimulation applied in A-G, bipolar stimulation in H, no stimulation (spontaneous action potential) in I.

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TTX concentration used in all experiments was 4 μg/ml (1.3 × 10⁻⁶M).

**Results**

**GENERAL CHARACTERISTICS OF CATECHOLAMINE-INDUCED SLOW RESPONSES IN OLD HEARTS**

**Graded Nature.**—The excitability of chick embryonic hearts (isolated ventricles, ages 9–19 days) was usually completely blocked by TTX, but the resting potential was generally not decreased by the TTX itself. The addition of catecholamines allowed slow graded responses to be produced in the presence of TTX (Fig. 1). The graded nature of the slow responses was shown by varying the intensity (Fig. 1C, F) or the duration (Fig. 1G) of the stimulating pulses. The slow responses had a higher threshold than did the normal action potential, and the maximum response was elicited at a current intensity two to ten times that required for the normal action potential. The largest responses were overshooting and resembled the plateau portion of the normal action potential (compare F with D). Double responses were sometimes observed (Fig. 1H): the first response of the pair appeared to be due to direct stimulation of the impaled cell, whereas the second response was due to propagation to the cell. Sometimes a double response summated.

**Spontaneity, Variability, and Time Course.**—In a few ventricles, the slow potentials

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**FIGURE 2**

Illustration of the gradual appearance of the catecholamine-induced graded response in ventricular cells of three chick hearts (12–17 days old). The records in each row were obtained from one cell; stimulus parameters were kept constant (5 msec rectangular pulses). First Column: Absence of normal action potential in the presence of TTX (4 μg/ml); shock artifacts (Art, arrows) only are present. Second Column: Responses elicited soon (1–2 minutes) after addition of catecholamines (1 × 10⁻⁶M; Epi = epinephrine; Iso = isoproterenol). Several superimposed responses are shown in E and H to illustrate their buildup in magnitude and duration. Third Column: Maximum responses elicited in the three cells (2–3 minutes after catecholamine addition). Note the similarity of the responses in F and I to the plateau of a normal action potential. Bipolar stimulation throughout.

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induced by exogenous catecholamines occurred spontaneously without electrical stimulation (Fig. 11). These slow potentials propagated throughout much of the myocardium and produced synchronous contraction. The mechanism of the automaticity inherent in some cells is not known, but it may be related to the spontaneous production of divalent cation spikes (Sr²⁺, Ba²⁺, Ca²⁺) in many myocardial cells (14-18).

The catecholamine-induced slow responses were often variable in magnitude and duration from one region of the ventricle to another. Sometimes, some regions of the ventricle did not respond electrically or show a visible contraction when viewed with the microscope. Cells within one region sometimes displayed nonuniform responses, particularly when bipolar stimulation was used and activation of the impaled cells was by propagation. In a few cases, the same cell showed variable responses which alternated in size. Thus, there appeared to be decremental propagation of graded responses. Estimates of the velocity of propagation of the slow response ranged from 0.5 to 10 cm/sec, and the maximal rate of rise usually fell in the range of 1 to 10 v/sec; the comparable values for the normal action potential in hearts of the same age are 40 cm/sec and 75-150 v/sec.

The slow responses began to appear rapidly (1-2 minutes) after addition of the catecholamines, and the maximum responses usually occurred within 2-5 minutes (Fig 2). There was a gradual increase in magnitude of the response at constant stimulus parameters, and this increase further demonstrates the graded nature of the response. The lag may be due in part to a buildup of catecholamine concentration in the vicinity of the impaled cell (interstitital fluid) by diffusion. Again note the similarity of the maximum responses to the plateau of the normal cardiac action potential (Fig. 2F, 1). With time (15-45 minutes), the slow responses began to diminish, and they eventually disappeared, probably concomitant with chemical conversion of the added catecholamines into inactive products.

The maximum size and duration of the slow response was dependent on the catecholamine concentration, as illustrated in Figure 3. In the same heart, the duration of the response increased as the dose was increased above 10⁻⁷M (left). The right of Figure 3 shows the records obtained from cells far from the

FIGURE 3
Illustration of the dose dependency of isoproterenol (ISOPROT)-induced responses in one heart (17 days old). The normal responses in Ringer's solution and the TTX blockade are not shown. Records for three different doses of isoproterenol (1 x 10⁻⁷, 1 x 10⁻⁸, and 1 x 10⁻⁹M), are illustrated in each row. Stimulus parameters were kept constant (5-msec pulses). First Column: Records obtained from impalements close to (−2 mm) the stimulating electrodes (bipolar). Note the increase in response duration with increase in dose. The artifact (Art) obscures the rising phase; before addition of catecholamine, the artifact was similar in appearance. Second Column: Records obtained from cells far (−5 mm) from the stimulating electrodes. The responses from the distal cells (especially in F) did not arise directly out of the stimulus artifacts (arrows), thus indicating propagation to the impaled cell. The smaller response in B (compared with that in A) illustrates the decremental (and variable) propagation which often occurred, especially at low doses.
stimulating electrode at each dose level; in these cases, the response did not arise directly out of the shock artifact, thus indicating propagation to the impaled cell.

Inactivation of the Fast Sodium Channels by Elevated External Potassium.—The catecholamine-induced responses also occurred when the fast Na⁺ channels were inactivated by elevation of [K⁺]o (e.g., to 20 mM) rather than by addition of TTX (Fig. 4). There were no differences in results when normal excitability was abolished by TTX or by elevated

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**FIGURE 4**

Catecholamine-induced responses in hearts in which the fast Na⁺ channels were inactivated by elevated [K⁺]o. A: Normal control action potential of a ventricular cell in a 15-day-old chick heart. B: Elevation of [K⁺]o to 20 mM caused excitability to fail completely even at a stimulus intensity tenfold greater than normal. C: Addition of epinephrine (Epi) (1 × 10⁻⁵ M) soon (1 minute) led to the appearance of slow electrical (and mechanical, not shown) responses which had a threshold higher than the normal action potential. The response is smaller because of the partial depolarization. Bipolar stimulation throughout.

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**FIGURE 5**

Experiment illustrating the inactivation potential for the catecholamine-induced slow responses by progressive depolarization in elevated [K⁺]o. Control action potential in normal solution and TTX blockade are not shown. A: Slow response induced by 1 × 10⁻⁵ M epinephrine in the presence of TTX (4 μg/ml) in normal [K⁺]o (3 mM). B-D: The responses were markedly reduced when [K⁺]o was increased to 40 mM (B) and 50 mM (C), and they entirely disappeared in 60 mM K⁺ (D). The inactivation potential for this heart was between —23 mV and —14 mV. Field stimulation used throughout.

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[K+]o, and many of the experiments for ascertaining the characteristics of the catecholamine-induced slow responses were repeated using the method of elevated [K+]o.

**Inactivation Potential for the Slow Response.**—The dependence of the size of the catecholamine-induced response on membrane potential was determined by elevation of [K+]o (to 10, 20, 30, 40, 50, and 60 mM). The magnitude and the duration of the slow response were progressively reduced with increasing depolarization (Fig. 5), and all responses disappeared at a [K+]o of 50 or 60 mM, corresponding to a membrane potential of −25 to −10 mv (Fig. 5D). Thus, the slow channels responsible for the slow electrical responses become partially inactivated as membrane potential is reduced and become completely inactivated at about −20 mv. A low inactivation potential is characteristic of slow cation (Na+ or Ca2+) channels (4, 10, 14,16, 19) and compares with an inactivation potential of −50 to −60 mv for fast Na+ channels.

**Evidence that the Catecholamine-Induced Slow Channel Admits Calcium**

Blockade by Lanthanum and Manganese.—The catecholamine-induced slow responses were rapidly and completely blocked by 1 mM La3+ (Fig. 6) or Mn2+ (Fig. 7A–F). Such concentrations generally had little or no effect on the +Vmax of the normal action potential (which presumably reflects the fast Na+ channels) but slightly depressed the plateau or slow-wave component (Fig. 7G, H). At a concentration of 1 mM, La3+ and Mn2+ are known to selectively block slow Ca2+ channels, i.e., they do not block slow Na+ channels (4). At higher concentrations, these heavy metal cations exerted a greater and more

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\text{FIGURE 6}
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La3+ blockade of the catecholamine-induced electrical and mechanical activities. A: Control action potential and contraction (CONTRAC) recorded by a force transducer. B: TTX (4 μg/ml) completely abolished the normal electrical responses and contractions. C: Epinephrine (EPI) (10^{-5}M) induced slow responses and contractions. D: Slow responses and contractions were abolished by adding 1 mM La3+. Field stimulation throughout.
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Blockade of the catecholamine-induced responses by Mn²⁺. A–C: An 11-day heart. A: Epinephrine (Epi)-induced response in the presence of TTX (4 µg/ml). B and C: Rapid depression (B, 2 minutes) and disappearance (C, 6 minutes) of the slow response produced by Mn²⁺ (1 mM). D–F: A 15-day heart. D: Epinephrine-induced response in 40 mEq [K⁺]₀ to inactivate the fast Na⁺ channels. E and F: Rapid depression (E, 2 minutes) and disappearance (F, 5 minutes) of the slow response caused by Mn²⁺ (1 mM). G–I: A 14-day heart, to show the effect of Mn²⁺ on the normal action potential. G: Control action potential. H: Mn²⁺ (1 mM) depressed the rate of rise of the overshoot or plateau portion of the action potential but did not abolish the response. I: Mn²⁺ (2 mM) further depressed the peak overshoot level. Field stimulation applied in A–C, bipolar stimulation in D–I.

Dependence on External Calcium Levels.—The occurrence of a slow response induced by catecholamines depended on extracellular Ca²⁺. For example, in the experiment illustrated in Figure 8A–D, addition of EGTA to reduce [Ca²⁺]₀ to low levels (<10⁻⁸ M) rapidly inhibited a previously induced catecholamine response (Fig. 8C). The response rapidly reappeared on addition of more CaCl₂ to return the level of free Ca²⁺ to the control value of 2 mM (Fig. 8D). When [Ca²⁺]₀ was reduced to near zero before addition of the catecholamine, no response occurred (Fig. 8E); however, the addition of Ca²⁺ nearly immediately allowed a response to be elicited (Fig. 8F).

Because of the variability of the response, it was difficult to quantify the relationship between peak overshoot, rate of rise, or duration of the slow response as a function of log [Ca²⁺]₀.

In an attempt to further demonstrate that the catecholamine-induced slow responses resulted from inward Ca²⁺ current, many experiments were done in which [Na⁺]₀ was lowered or reduced to zero by substitution with Li⁺, choline⁺, or sucrose. However, in

nonspecific depressant effect (Fig. 7I). Ruthenium red also completely blocked the catecholamine-induced contractions when applied at a concentration of 1 mM, but its action may be nonspecific.

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Dependence of the catecholamine-induced response on extracellular Ca$^{2+}$, $[\text{Ca}^{2+}]_0$, was lowered either after induction of the response (A–D) or before addition of catecholamine (E, F). A: Control action potential in normal Ringer’s solution ($[\text{Ca}^{2+}]_0 = 2$ mM). Abolition of these responses by TTX (4 ng/ml) is not shown. B: Impalement of another cell showing large slow response after addition of epinephrine (Epi) ($1 \times 10^{-6}$ M). C: Addition of EGTA (2 mM) to decrease $[\text{Ca}^{2+}]_0$ to zero led to rapid disappearance of the responses. D: Addition of CaCl$_2$ to give 2 mM allowed large responses to occur again. E: Epinephrine ($1 \times 10^{-6}$ M) did not lead to slow responses in the absence of extracellular Ca$^{2+}$ in this heart blocked by TTX (4 $\mu$g/ml). F: Slow responses rapidly occurred after addition of 2 mM Ca$^{2+}$. Bipolar stimulation used in A–D, field stimulation in E and F.

Ryanodine, a compound which reverses digitalis-induced arrhythmias and depresses Ca$^{2+}$ release from the sarcoplasmic reticulum (20), had no effect on the catecholamine-induced responses. This compound in high concentrations (up to $2 \times 10^{-5}$ M) did not depress or prevent the catecholamine effect. Hence, ryanodine does not block sarcolemmal Ca$^{2+}$-channels of chick myocardial cells.

Relationship to Contraction: Substitution of Barium or Strontium.—As stated above, contractions appeared concomitantly with the catecholamine-induced slow electrical responses (Fig. 6), and regions of some hearts which did not exhibit slow potentials also did not exhibit contractions. In all cases, there appeared to be a close correlation between the slow response and contraction, and, whenever the electrical responses were larger or longer in duration, the contractions appeared to be more forceful. The contractions were graded with the dose of catecholamines (Fig. 9). In this case, the maximal contraction occurred between 1 and $5 \times 10^{-6}$ M isoproterenol. The contractions were also dependent on extracellular Ca$^{2+}$ levels. The isoproterenol-induced contractions in the presence of TTX were considerably diminished when $[\text{Ca}^{2+}]_0$ was reduced to nearly zero by adding an equimolar amount of EGTA. This diminished contraction was rapidly restored by addition of Sr$^{2+}$ or Ba$^{2+}$. These results suggest that Sr$^{2+}$ and Ba$^{2+}$ can substitute for Ca$^{2+}$ as the current carrier for the catecholamine-induced slow response. La$^{3+}$, Mn$^{2+}$, and ruthenium red, agents which depressed or blocked the electrical responses, also depressed the contractions. In some cases, La$^{3+}$ only partially depressed the contractions but did not completely block them except over a long period; this observation could be due to the field stimulation causing release of Ca$^{2+}$ directly from the sarcoplasmic reticulum (21). Reduction of $[\text{Ca}^{2+}]_0$ to 1 mM (from its normal level of 2 mM) by addition of 1 mM EGTA did not greatly affect the normal contraction but did greatly diminish the catecholamine-induced contraction. When $[\text{Ca}^{2+}]_0$ was reduced nearly to zero by adding

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Mechanical records from one heart (17 days old) illustrating the dose dependency of the magnitude of contraction obtained with the catecholamine-induced response. A: Control contractions in normal Ringer's solution (left portion) are rapidly inhibited by addition of TTX (4 μg/ml, at arrow). B-D: Stimulus intensity was increased tenfold above the normal level used in A (constant duration of 5 msec). Addition of isoproterenol (ISO) (1 × 10⁻⁷M, at arrow) in B produced a rapid buildup of contractions (C, D); the record shown in D taken at 5 minutes was the maximum response for that dose. E: Maximum response obtained at 3 minutes after increase in isoproterenol concentration to 5 × 10⁻⁷M. Note that these responses are larger than control in A. F: No further increase in response at 5 minutes after elevation to 1 × 10⁻⁶M isoproterenol. Field stimulation used.

2 mM EGTA, the catecholamine-induced contraction sometimes diminished more rapidly than the normal contraction. If this observation is valid, the catecholamine-induced contraction may be more directly dependent on [Ca²⁺]₀ than is the normal contraction. To determine whether there is any difference between the dependencies on [Ca²⁺]₀ of these two types of contractions, perfusion experiments should be done to minimize the problem of diffusion through the myocardium. Mn²⁺ and La⁺⁺ (1 mM) only partially depressed the normal contraction, and catecholamines still had a large positive inotropic effect.

RELATIONSHIP OF THE CATECHOLAMINE-INDUCED RESPONSE TO THE NORMAL ACTION POTENTIAL PLATEAU

Similarity of Shapes.—As stated above, in many instances, the maximum catecholamine-induced slow response had a shape, duration, and peak overshoot value very similar to that of the plateau of the normal action potential. This fact is evident in many of the figures, including Figures 1F, 2F and I, 4C, 5A, and 7A.

Effect of Manganese, Lanthanum, and Low External Calcium.—Also as stated above, Mn²⁺ and La⁺⁺ tended to depress the plateau component and peak overshoot of the normal action potential (Fig. 7G-1). The peak overshoot of the cardiac action potential represents inward Ca²⁺ current, whereas the rapid upstroke represents Na⁺ current (19, 22). Thus, one current which contributes to the normal plateau is Ca²⁺ current. However, the plateau component did not disappear in nearly Ca²⁺-free solution. The duration (50% and 90%) of the action potential was reduced...
Illustration of slow responses which sometimes persisted in the presence of TTX without addition of exogenous catecholamines. A and B: A 9-day-old heart. A: Control action potential in normal solution. B: Record taken from another impalement 5 minutes after addition of TTX (8 μg/ml) and elevation of [Ca$^{2+}$]$_o$ to 4 mM. C: A 12-day-old heart. Record taken 10 minutes after addition of TTX (4 μg/ml) ([Ca$^{2+}$]$_o$ = 2 mM). Note the similarity of these persisting slow responses with the catecholamine-induced responses shown in other figures. Bipolar stimulation used.

Occasional Persistence of Slow Responses in the Presence of Tetrodotoxin.—There was an occasional persistence of slow electrical responses (and contractions) in the presence of TTX but in the absence of any added exogenous catecholamine (Fig. 10). That is, in a few cases (Table 1), TTX failed to completely block excitability. These persisting responses resembled the catecholamine-induced responses in shape and in other characteristics. They were slow rising, had a higher threshold than the normal response, and were graded. These slow responses were also propagated decrementally, allowing synchronous contraction of much of the ventricle. Elevation of [Ca$^{2+}$]$_o$ increased the size of the response and the probability of its occurrence (Fig. 10B). Increased stretch of the tissue also appeared to increase the likelihood of its occurrence.

Effect of Temperature.—The effect of cooling on the isoproterenol-induced slow response seemed to parallel that on the normal action potential plateau (Fig. 11). Rapid cooling from 37°C to 27°C produced a pronounced increase in the duration of the catecholamine-induced response (Fig. 11C, D). The percent increase in duration was similar to that produced by the same degree of cooling on the normal action potential (heart driven at constant rate) (Fig. 11E, F).

### Table 1

| Embryonic age (days) | Fraction of hearts showing persisting response
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<tr>
<td>9–13</td>
<td>11/19 (68%)</td>
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<tr>
<td>14–19</td>
<td>6/57 (11%)</td>
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<tr>
<td>Mechanical Response Only†</td>
<td>13/38 (34%)</td>
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*Denominator of fraction gives the total number of hearts examined. In almost all cases (only 2 exceptions), TTX (4 μg/ml) caused complete failure of excitability to normal electrical stimulation. However, some hearts gave a slow response, resembling the catecholamine-induced response, to stimulation ten times that normally required (constant rectangular pulse duration of 5 msec); these hearts are categorized as having a persisting response.

In these experiments, contractions only were recorded. The higher incidence of persisting contractions may be due to field stimulation causing direct release of Ca$^{2+}$ from the sarcoplasmic reticulum of many of the cells.
Cooling produced a pronounced increase in duration of the catecholamine-induced slow response. A–D: An 18-day-old heart. A: Control action potential in normal Ringer's solution at 37°C. B: Record taken from another cell showing abolished excitability after addition of TTX (4 μg/ml). C: Addition of isoproterenol (ISO) (1 × 10⁻⁴M) produced the usual slow responses. D: Suddenly lowering the temperature to 27°C, by rapidly changing the bath with a solution identical to that in C except 10°C cooler, caused a marked prolongation of the slow response. Broken curve is the superimposed response from C. E and F: A 17-day-old heart driven electrically at 1/sec. The normal action potential at 37°C (E) was greatly prolonged when the bath temperature was suddenly lowered 10°C (F). Note the similarity of the effect of temperature on the catecholamine-induced slow response and the normal action potential. Bipolar stimulation throughout.

**ABSENCE OF AN EFFECT OF CATECHOLAMINES IN YOUNG HEARTS**

Epinephrine and isoproterenol, in concentrations as high as 1 × 10⁻⁴M and for periods up to 20 minutes, had no discernible effect on the electrical or mechanical activity of ventricular muscle from young chick embryos (3–5 days old). Sometimes there was a very slight increase in frequency of spontaneous beating (entire heart isolated for these young hearts). Some typical mechanical records are illustrated in Figure 12. Microelectrode penetrations indicated that there was no prominent effect of catecholamines on action potential shape or duration in the ventricular cells of young hearts. It appeared that the ability to respond to catecholamines, with respect to Ca²⁺ channels, was absent in young ventricular cells. In contrast, in old hearts, catecholamines had a large and rapid positive inotropic effect.

**PHARMACOLOGICAL CONSIDERATIONS**

**Structure-Activity Relationships.**—The relative effectiveness of the series of catecholamines and their precursors were determined, and the results are summarized in Table 2. Isoproterenol was the most effective agent, producing effects at concentrations as low as 1 × 10⁻⁴M. Epinephrine and norepinephrine...
Lack of effect of isoproterenol (10^{-5}M) on the contraction of three representative young embryonic hearts (3-5 days old). Contractions recorded on a penwriter using a piezoelectric cartridge; the recordings are diphasic because of the nature of the recording system. The vertical deflection was arbitrarily adjusted, but the amplifier gain was kept constant in each experiment. The contractions recorded by this method did not significantly change even 10 or 15 minutes after addition of isoproterenol. Spontaneous activity.

were the next most effective, followed by dopamine and dopa. Tyrosine, phenylalanine, and tyramine (one trial) were ineffective. Since the catecholamines were also effective on hearts 10-13 days old, presumably before functional adrenergic innervation has developed (23), the effective agents probably acted directly on the myocardial cells and not on the adrenergic neurons to release neurotransmitter. All the effective agents have a second hydroxyl group on the benzene ring at the number 3 carbon, thus suggesting that this group is a requirement for activity. The activity may be enhanced by the addition of a hydroxyl group on the first carbon of the sidechain and methyl groups on the amino nitrogen and by the absence of the carboxyl group.

Lack of Effects of Acetylcholine and Prostaglandins.—Acetylcholine (up to 2 \times 10^{-5}M)

---

**TABLE 2**

Relative Effectiveness of Various Catecholamines and Their Precursors in Producing Slow Electrical and Mechanical Responses in Tetrodotoxin-Blocked Older Chick Embryonic Hearts (10-19 Days Old)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (M)</th>
<th>Total number of hearts examined</th>
<th>Hearts giving a positive response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td>10^{-5}, 10^{-4}, 10^{-3}</td>
<td>39</td>
<td>35</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>10^{-5}, 10^{-4}</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>10^{-5}, 10^{-4}</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Dopamine</td>
<td>10^{-5}</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>l-Dopa</td>
<td>10^{-3}</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>l-Tyrosine</td>
<td>10^{-5}</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>l-Phenylalanine</td>
<td>10^{-4}</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

*The number of hearts which gave a slow electrical response. Ineffectiveness of an agent was only accepted when a subsequent dose of isoproterenol was positive. At embryonic ages of 10-19 days, the hearts are presumably innervated by the sympathetic fibers; the hearts aged 10-13 days presumably do not yet have functional sympathetic innervation. There were no consistent differences between these age groups.

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had no effect on the catecholamine-induced responses (Table 3). It neither mimicked, depressed, nor blocked the catecholamine effect. However, acetylcholine receptors are virtually absent in chick ventricular cells (4, 24). Prostaglandins E1 and F2α, in concentrations as high as 20 μg/ml, also had no effect on the catecholamine-induced responses (Table 3).

Effects of Cyclic Adenosine Monophosphate and Related Compounds.—Cyclic 3′,5′ AMP (C-AMP) and dibutyryl cyclic AMP (DC-AMP) over long time periods of 20–60 minutes often mimicked the catecholamine-induced effects, both electrically and mechanically (Fig. 13, Table 3). C-AMP and DC-AMP, when added together or separately, slowly led to the development of large contractions, either when preequilibrated with theophylline (Fig. 13A–F) or when [Ca^{2+}]_o was elevated (Fig. 13H–N). In elevated [Ca^{2+}]_o, with or without caffeine, DC-AMP often had a more rapid effect. The addition of the β-receptor blocking agent, dl-propranolol, reduced but did not abolish this action of C-AMP and DC-AMP (Fig. 13G). However, the slow response produced by isoproterenol was rapidly abolished by dl-propranolol (4 × 10^{-6} M) (Fig. 14, Table 3), in agreement with previously published reports (11, 13). Over short periods (up to 10 minutes), C-AMP and DC-AMP had no effect on the catecholamine-induced response; in high concentrations (up to 3 mM), they neither mimicked, potentiated, nor blocked the effect of the catecholamines (Fig. 15A–F). This lack of effect was true even with preequilibration with 1 mM theophylline (Fig. 15G–I). Theophylline also did not lower the threshold dose for the epinephrine effect.

Caffeine or theophylline partially mimicked the effect of the catecholamines but usually over a long time period (Fig. 16A–G). In a few cases, however, the addition of caffeine and theophylline rapidly led to a catecholamine-like effect (Fig. 16J). These methylxanthine-induced contractions were rapidly depressed by low Ca^{2+} (Fig. 16K), as were the catecholamine-induced contractions. In a few cases, the caffeine-induced contractions were small or absent, although the muscles were capable of rapidly giving large isoproterenol-induced contractions.

**Discussion**

The vertebrate cardiac action potential is composed of two components, the spike and the slow wave (plateau), which can be separated under various experimental conditions (25–27). Inward Ca^{2+} current plays an important role in the electrogensis of the late upstroke and the early part of the plateau of the normal cardiac action potential (12, 22, 28). Voltage-clamp experiments show a slow component of inward membrane current in addition to the fast Na^{+} current (19, 29–32). In some species, the slow current is carried primarily by Ca^{2+} and in others by both Ca^{2+} and Na^{+}. To clearly distinguish the existence of the slow current, it is usually necessary to suppress the fast Na^{+} current by (1) Na^{+}-free solutions, (2) TTX, or (3) inactivation of the fast Na^{+} system by depolarization using either conditioning clamp pulses or elevated [K^{+}]_o.

**TABLE 3**

Summary of Agents and Conditions Tested for an Effect on the Catecholamine-Induced Response

<table>
<thead>
<tr>
<th>Produced the response*</th>
<th>Partially mimicked the catecholamines</th>
<th>Blocked the response</th>
<th>Ineffective</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoproterenol</td>
<td>Cyclic 3′,5′ AMP</td>
<td>Propranolol</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Dibutyryl cyclic 3′,5′ AMP</td>
<td>La^{3+}</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Theophylline</td>
<td>Mn^{2+}</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>Dopamine</td>
<td>Caffeine</td>
<td>Ruthenium red</td>
<td>Prostaglandine</td>
</tr>
<tr>
<td>Dopa</td>
<td>Ca^{2+} (elevated)</td>
<td>Zero Ca^{2+}</td>
<td>Ryanodine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zero Na^{+}</td>
<td></td>
</tr>
</tbody>
</table>

*Cooling prolonged the response.
Long exposure to cyclic AMP (C-AMP) and dibutyryl cyclic AMP (DC-AMP) produced slow responses similar to catecholamine-induced responses. Two representative experiments are illustrated. The first record in each experiment gives the control contractions (A, H), and the second record gives the inhibition of contraction produced by TTX (4 µg/ml) (B, I). The insets in C–F give representative electrical recordings taken during each condition.

Experiment I: Theophylline (Theo) (0.5 mM) produced only a small positive inotropic effect and a small electrical response at 15 minutes (C). Addition of C-AMP and DC-AMP (2 mM each) slowly produced a large positive inotropic action with accompanying large electrical responses (D–F, 10 minutes, 20 minutes, and 1 hour). Note the similarity of the electrical responses to the usual catecholamine-induced response. Propranolol (Propran) (4 x 10⁻⁶ M) did not abolish these responses (G), in contrast to its rapid effect on the isoproterenol-induced response (Fig. 14). The decrease in the amplitude of contraction by propranolol may be due to a direct partial inhibitory local anestheticlike effect on the electrical response.

Experiment II: In the presence of high [Ca²⁺]₀ (4 mM), DC-AMP (2 mM) alone slowly allowed the development of contractions with accompanying electrical responses (not shown) (J–N, 5 to 30 minutes). Bipolar stimulation throughout.

The present results clearly demonstrated that catecholamines rapidly induced slow electrical responses in chick embryonic myocardial cells in which the fast Na⁺ channels were inactivated by either TTX or elevated [K⁺]₀. These slow overshooting responses had a shape similar to that of the plateau component of the normal action potential, and cooling prolonged both to about the same extent. Several lines of evidence showed that the slow response resulted from activation of a kinetically slow channel (compared with the

### Figure 13

Antagonism of the catecholamine-induced response by a β-receptor blocking agent, dl-propranolol (Propran) (4 x 10⁻⁶ M). Control action potential and its abolition by TTX are not shown. A: Isoproterenol-induced response in presence of TTX (4 µg/ml). B and C: Rapid depression (B, at 1 minute) and abolition (C, at 2 minutes) of response by propranolol. Bipolar stimulation used.

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Cyclic AMP (C-AMP) and related agents in short time periods up to 10 minutes did not mimic the catecholamine effect. Three experiments depicted on three different hearts. Control action potentials are not shown. First Column: Excitability abolished by TTX (4 μg/ml). Second Column: 5-10 minutes after addition of cyclic AMP or related agents. Cyclic AMP (2 mM, B) and dibutyryl cyclic AMP (DC-AMP) (3 mM, E) had no effect. Theophylline (Theo) (1 mM, H) had no effect by itself (10-minute period) or after addition of DC-AMP (3 mM); record taken 10 minutes after DC-AMP addition. Third Column: Responses obtained 2 minutes after addition of epinephrine (Epi) (1 X 10⁻⁵ M). Bipolar stimulation throughout.

FIGURE 15

CATECHOLAMINE-INDUCED CALCIUM CHANNELS

The evidence for an inward Ca²⁺ current participating in the catecholamine-induced slow responses may be summarized as follows. (1) The slow responses were rapidly and completely blocked by La³⁺ and Mn²⁺ (at 1 mM), agents known to block Ca²⁺ current. However, since Mn²⁺ also blocks slow Ca²⁺-Na⁺ channels (2, 10), it is difficult from this finding alone to exclude the participation of some Na⁺ current. However, 1 mM Mn²⁺ (or La³⁺) does not block the slow Na⁺ channel present in young embryonic ventricular cells (3, 4) or cultured heart cells (7). Therefore, if
Caffeine (Caff) and theophylline mimicked the effect of the catecholamines, usually slowly but sometimes very rapidly. Two experiments are depicted in two different hearts using caffeine. The first record in each experiment gives the control contraction (A, H), and the second gives the inhibition of contraction produced by TTX (4 µg/ml) (B, I) The insets in B and D-G give representative electrical recordings taken during each condition. Experiment I: Caffeine (2 mM) slowly induced large contractions and electrical responses (C-G, 3-30 minutes). Experiment II: Caffeine (2 mM) rapidly induced large contractions (J) which were depressed and abolished within 1 minute when [Ca²⁺]₀ was lowered to nearly zero by addition of 2 mM ECTA (K). Caffeine and theophylline sometimes were only slightly effective or completely ineffective even after long periods of 20-25 minutes. Bipolar stimulation throughout.

Voltage-clamp experiments on frog atrium indicate that epinephrine activates a Mn²⁺-sensitive slow channel through which both Ca²⁺ and Na⁺ can pass; this finding could partly explain epinephrine's positive inotropic action and its ability to restore impaired conduction (10). Epinephrine (maximal response at 1.1 x 10⁻⁶M and half-maximal at 0.5 x 10⁻⁶M) produces propagating plateau-like potentials in cow Purkinje fibers in 16 mM [K⁺]₀ which are blocked by Mn²⁺ and β-receptor blocking agents (11). Since the amplitude of the slow response changes by only 17 mv/tenfold change in [Ca²⁺]₀, a participation of Na⁺ current or a decrease in potassium conductance is not ruled out. Sr²⁺ is able to substitute for Ca²⁺. Catecholamines restore propagating potentials and contractions in guinea pig atrium in 22 mM [K⁺]₀ which are blocked by Mn²⁺ and propranolol (13). Their peak overshoots vary the theoretical 28 mv/tenfold change in [Ca²⁺]₀; in reasonable agreement with the present results, they fail at a membrane potential of −30 mv. The threshold concentrations averaged about 0.7 x 10⁻⁶M for isoproterenol, 3 x 10⁻⁷M.
for epinephrine, and $1 \times 10^{-6}$ M for norepinephrine, and the maximum response occurs at about double the threshold concentration (13). Ba$^{2+}$, Sr$^{2+}$, or elevated Ca$^{2+}$ produce similar responses. The effect of catecholamines on the level of phosphorylase $a$, possibly mediated by changes in [Ca$^{2+}$], is also blocked by La$^{3+}$ (33).

Thus, it appears that catecholamines make kinetically slow channels available in the chick through which Ca$^{2+}$ can pass. In some membranes (frog atrium), the TTX-insensitive slow channel admits both Ca$^{2+}$ and Na$^{+}$ (29), whereas in other membranes (e.g., rabbit heart) the slow channel admits only Ca$^{2+}$ (34). The divalent cations, Sr$^{2+}$ and Ba$^{2+}$, appear to be able to pass through the same channel as Ca$^{2+}$. Since the unhydrated radii of Ca$^{2+}$ and Na$^{+}$ are nearly the same, whereas the hydrated radius of Ca$^{2+}$ is considerably larger, it appears that the catecholamines do not open up simple pores in the membrane. That is, the Ca$^{2+}$ channels made available by the catecholamines must have specificity; perhaps a carrier molecule or divalent cation ionophore is made available.

The catecholamines may exert their actions through binding to $\beta$-receptor sites, since propranolol blocks the responses (11, 13). It is not known whether one catecholamine molecule bound to one receptor site makes one Ca$^{2+}$ channel available. The fact that cyclic 3',5' AMP, dibutyryl cyclic AMP, theophylline, and caffeine usually slowly mimicked much of the action of the catecholamines leaves open the possibility that the catecholamine action is mediated through adenyl cyclase and an increase in internal levels of cyclic AMP. Caffeine (1-5 mM) also mimicks the catecholamines in cow Purkinje fibers (11). A crude estimate of the density of Ca$^{2+}$ channels at peak activation is 1 channel/3 $\mu m^2$. This value compares with an estimated surface density of Na$^{+}$ channels during peak activation of nerve membrane of about 30 Na$^{+}$ channels/$\mu m^2$ (1), of K$^{+}$ channels in resting myocardial cells of <1 K$^{+}$ channel/$\mu m^2$ (35), and of Na$^{+}$-K$^{+}$ pump sites of $\sim$ 1,000/$\mu m^2$ (36). We do not know when $\beta$-receptors first appear in the heart during development. Although it has been reported that catecholamines exert a positive inotropic action on chick embryonic hearts that are only 4 days old (37), we did not find that young hearts (2-5 days old) responded mechanically to catecholamines. Thus, the ability of catecholamines to induce Ca$^{2+}$ channels may first arise after day 5 and before day 9, or about the same time that fast Na$^{+}$ channels are becoming available (3, 4, 7). Catecholamine-containing neurons are not observed until about day 16 (23). Since the young hearts were not blocked by TTX and since the catecholamine-induced response did not occur in the complete absence of external Na$^{+}$ in the old hearts, it was not possible to unequivocally determine whether the catecholamines had no effect on inducing slow Ca$^{2+}$ channels in young hearts. In general, the catecholamines did not appear to affect the shape of the normal action potential in the young heart. Others have reported that catecholamines increase the amplitude and the duration of the plateau phase of the cow Purkinje (11) and frog atrial (10) action potentials.

It is possible that the inward Ca$^{2+}$ current ($I_{ca}$) flowing during the slow catecholamine-induced response is directly responsible for activation of the contractile elements. Assuming a membrane capacitance ($C_m$) of $5 \times 10^{-9}$ farads/cm$^2$ and a ramp rise ($dV/dt$) of the slow response averaging 5 v/sec, then the minimum membrane current which must flow (the capacitive current, $I_c = C_m \cdot dV/dt$) to produce the response is 25 $\mu$amp/cm$^2$ (compared with 750 $\mu$amp/cm$^2$ for the normal action potential). (Since the action current associated with the slow response is very small, this poses interesting questions as to how it propagates from cell to cell.) This current, if carried only by divalent ions, corresponds to about $13 \times 10^{-11}$ moles/sec cm$^{-2}$. However, the total current is the sum of the capacitive and the ionic currents, the latter usually being considerably greater than the former. An estimate of the minimum Ca$^{2+}$ influx necessary to give peak tension development, i.e., complete saturation of the Ca$^{2+}$...
binding sites on the contractile proteins and elevation of \([Ca^{2+}]_i\), from \(1 \times 10^{-7}\) to \(1 \times 10^{-5}\text{M}\), is \(60 \times 10^{-9}\text{ moles/g}\) (38). If 1 g is equivalent to 3.3 \(\times 10^2\text{ cm}^2\), on the basis of cylindrical cells 10\(\mu\) in diameter and 150\(\mu\) long and a total extramyocardial cell space of 20\%, the minimum \(Ca^{2+}\) influx required becomes 18.1 \(\times 10^{-12}\text{ moles/cm}^2\). For a cell surface area of 4.7 \(\times 10^{-4}\text{ cm}^2\), the minimum \(Ca^{2+}\) influx per myocardial cell is 36.2 \(\times 10^{-11}\text{ moles/beat}\). For a cell surface area of 4.7 \(\times 10^{-6}\text{ cm}^2\), the minimum \(Ca^{2+}\) influx per myocardial cell is 85.5 \(\times 10^{-17}\text{ moles/sec cell}\) or 36.2 \(\times 10^{-14}\text{ moles/sec cm}^{-2}\). This value is only about threefold larger than the capacitive current estimation. Therefore, the contraction which accompanies the catecholamine-induced slow response may be nearly completely accounted for by the \(Ca^{2+}\) current without the necessity of invoking the trigger \(Ca^{2+}\) hypothesis, i.e., that \(Ca^{2+}\) influx produces further release of \(Ca^{2+}\) from the sarcoplasmic reticulum. The electrochemical driving force (\(E_m - E_{oa}\)) for inward \(I_{Ca}\) across the resting membrane is about 210 mv. Since chick myocardial cells do not have transverse (T) tubules (39-41), the catecholamine-induced \(Ca^{2+}\) channels must be in the surface sarclemmal membrane.

Although the \(Ca^{2+}\) influx across the sarclemma during the catecholamine-induced responses may be sufficient to account for activation of the contractile elements, in the normal action potential it is uncertain whether a second \(Ca^{2+}\) pool is utilized, namely, \(Ca^{2+}\) influx from the lumen of the sarcoplasmic reticulum to the myoplasm across the sarcoplasmic reticulum membrane. \(La^{3+}\) or \(Mn^{2+}\) reduced, but did not completely suppress, the normal contractions. It has been reported that, in perfused rat hearts, 1 \(\mu\)M \(La^{3+}\) also only partially depresses the contractions (although 10 \(\mu\)M \(La^{3+}\) completely suppresses contraction) (33). Since no consistent differences in the time constant of diminution of contraction following step changes in \([Ca^{2+}]_o\) were observed during the normal action potential and during the catecholamine-induced response, this parameter does not enable distinction between the two possible \(Ca^{2+}\) sources. However, the problem of diffusion through the interstitial space may obscure possible differences. If a second pool of \(Ca^{2+}\) is involved, then somehow the sarcolemmal membrane potential change must signal the release of \(Ca^{2+}\) from the sarcoplasmic reticulum. Since there is no evidence that the sarcoplasmic reticulum of cardiac muscle is open to the interstitial fluid (40, 41), in contrast to the sarcoplasmic reticulum (terminal cisternae) of skeletal muscle (42), it is unlikely that the change in \(E_m\) directly leads to a change in \(E_{SB}\) by some form of electrical continuity.

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CATECHOLAMINE-INDUCED CALCIUM CHANNELS


Calcium Current Channels Induced by Catecholamines in Chick Embryonic Hearts Whose Fast Sodium Channels are Blocked by Tetrodotoxin or Elevated Potassium

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