Electrical Responses of Guinea Pig Coronary Artery to Transmural Stimulation

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Isolated segments of the guinea pig circumflex coronary artery were stimulated transmurally with brief duration (0.05 msec) pulses while recording intracellularly. Resting membrane potential was $-60.3 \pm 4.3$ mV ($n = 66$). Transient depolarizations (TDs) graded in amplitude with stimulus intensity were elicited with single stimuli. An action potential of $47 \pm 5$ mV was superimposed upon the TD above a threshold membrane potential of $-48 \pm 4$ mV. Following the spike, a period of slow repolarization requiring $77 \pm 33$ seconds was observed. Repetitive stimulation led to summation of TDs and a slow depolarization ($\leq 21$ mV) that persisted for up to 90 seconds. The TD was reduced in amplitude when the membrane was depolarized with increased $[K]$, and enhanced when the membrane was hyperpolarized by adding potassium back to a potassium-depleted tissue. Phentolamine (10$^{-7}$ M), prazosin (10$^{-6}$ M), guanethidine (2 x 10$^{-5}$ M), $\alpha, \beta$-methylene ATP (2 x 10$^{-5}$ M), tetrodotoxin (10$^{-5}$ M), and 10–20 mM norepinephrine did not block TDs, action potentials, or slow depolarization. TDs were abolished following cold storage (4°C) for 4 days and significantly reduced in a 2.25-mM CdCl$_2$ solution with 0.25 mM CaCl$_2$. Acetylcholine (10$^{-4}$ M to 10$^{-1}$ M) produced membrane hyperpolarization and reduced the amplitude of TDs and altered their time course. Atropine (10$^{-3}$ M) blocked the effects of acetylcholine but had no effect on the response to nerve stimulation. These observations indicate that TDs exhibit some, but not all, of the properties characteristic of excitatory junction potentials obtained with release of neurotransmitter substances in other vessels. If these are neural events, they must involve a transmitter other than norepinephrine, acetylcholine, or ATP. (Circulation Research 1988;62:585–595)
free, taking care to never directly press on or stretch the artery. Vessels ranged in diameter from 250 to 500 μm. Hematoxylin and eosin staining revealed a media containing four to six smooth muscle cell layers and an intact endothelium (n = 3).

The preparation was immersed in 37°C oxygenated Krebs solution in a 4-ml bath and superfused at 6 ml/min. It was pinned at the distal end and cannulated at the proximal end with a glass pipette having an outer tip diameter of 150 μm. The cannula was tied in place with a human hair. A perfusion pressure of 10 mm Hg was applied to the vessel from a gravity driven perfusion system attached to the cannula. This pressure was selected since it adequately distended the vessel (i.e., diameter changed by less than 3% for pressures greater than 10 mm Hg and less than 80 mm Hg). The leakage was approximately 1–2 ml/hour except in experiments with low calcium solution in which the leak was reduced to zero. Albumin (1.5 g/100 ml) was included in the perfusate since it is thought to protect the endothelial lining26 and enhance the viability of the preparation.17 Platinum electrodes (0.5-mm diameter) were placed on either side of the isolated vessel approximately 300 μm away from the vessel wall. A Grass stimulator was used to deliver stimulus pulses (model S88, Grass Instrument, Quincy, Massachusetts). Stimulus voltages were measured at the output of the stimulus isolation unit with the electrodes in parallel in the bath. For all experiments unless otherwise specified a stimulus duration of 0.05 msec was used. To record from the intimal side of the media the preparation was cut open and pinned with the adventitial side against the sylgard base. The stimulating electrodes were placed on either side of the strip, and cells were impaled through the intima.

For experiments in which the vessels were denervated by cold storage, the coronary artery was dissected from the heart and stored at 2°C in Krebs solution in a closed glass container for 4 days. One and one-half hours equilibration time at 37°C was allowed before beginning experiments. Stimulation and recording from the cold-stored vessels were conducted in a manner identical to fresh vessels.

For experiments with CdCl₂, Krebs bicarbonate solution was substituted with HEPES-buffered solution (mM: 20 HEPES; 123.5 NaCl, 4.7 KCl, 1.2 MgCl₂, 2.5 CaCl₂, 5.5 dextrose) 30 minutes prior to beginning experiments. The pH was adjusted to 7.4 with NaOH, and the solution was aerated with 100% O₂. Switching to HEPES solution resulted in a transient depolarization of approximately 6 mV and a slight enhancement of EJP amplitude. After 30 minutes, the membrane potential had returned to within 3 mV of control, and the EJP was no longer enhanced. Low calcium solution with CdCl₂ was made by substituting 2.25 mM CaCl₂ in HEPES-buffered solution for an equimolar concentration of CdCl₂.

Glass microelectrodes filled with 3 M KCl and having resistances between 60 and 120 MΩ were used. Impalements were made through the adventitia in a portion of the vessel that was between the poles of the stimulating electrodes unless otherwise specified. Impalements were judged on the basis of a rapid drop in potential upon entering the cell, a low noise level, minimal change in electrode resistance and zero potential before and after impalement, and by the presence of EJPs. In cold-stored vessels in which EJPs were absent, impalements were sometimes confirmed by increasing extracellular potassium concentration ([K]₀) to 26.1 mM for 5 minutes. The presence of depolarization was indicative of a "true" impalement. Individual impalements of at least 30 minutes were routinely obtained in this preparation. Signals were viewed on a digital oscilloscope (Nicolet, Madison, Wisconsin) and stored on magnetic tape with an FM tape recorder (Hewlett-Packard, Palo Alto, California) for later playback.

For experiments with the inferior mesenteric artery, the descending colon with its attached mesentery and aorta were removed from the guinea pig. After pinning out the aorta and mesenteric sheath, the colon was removed. The mesenteric artery was cannulated at its origin at the aorta, pinned distally to obtain a preparation of equal length to the coronary artery, and inflated with 10 mm Hg distending pressure. Impalements were made through the adventitia.

The time constant of transient depolarizations (TDs) was measured using a computer and custom-designed software that calculated the best-fit line through all points of a selected portion of the declining phase of the TD when plotted logarithmically. Duration of the TD was the time from the beginning of the depolarization until the potential returned to 95% of the former resting potential.

Statistical significance was determined by two tailed paired or unpaired t test. The changes were considered significant at p < 0.05. Data are expressed as mean ± SD. The n values refer to the number of preparations.

The following drugs were used: prazosin HCl (Pfizer,); phenolamine mesylate (Regitine; Ciba-Geigy, Summit, New Jersey); noradrenaline HCl (NE; Sigma Chemical, St. Louis, Missouri); acetylcholine chloride (ACh; Sigma); atropine sulphate (Sigma); tetrodotoxin (TTX; Calbiochem, San Diego, California); α,β-methylene adenosine 5'-triphosphate (Sigma); guanethidine sulphate (Ciba); and HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; Sigma). Prazosin (10⁻⁷ M) stock was made immediately before beginning the experiment by dissolving prazosin in warmed distilled water and then diluting in Krebs bicarbonate solution to obtain a final concentration of 10⁻⁶ M.

Results
Response to Transmural Stimulation With Single Stimuli

The smooth muscle membrane of the guinea pig coronary artery was electrically quiescent, and the resting membrane potential was −60.3 ± 4.3 mV (n = 66; 308 cells). Transmural nerve stimulation (TMS) resulted in TDs. These depolarizing responses
occurred regardless of which stimulating electrode was selected as the cathode. Under no circumstances did a hyperpolarizing response occur with either single or multiple stimuli. The latency between the onset of stimulation and the beginning of the TD was typically 4–5 msec; however, in a few cells latencies as great as 20 msec were observed. The TD did not decline as a single exponential function. The initial decline during the first 300–700 msec after initiation of the TD could be represented by a straight line when plotted semi-logarithmically (see inset, Figure 1). The mean value of the slope ($\tau_f$) for this initial decline was 450 ± 139 msec ($n = 13$). After 300–700 msec the decline of the TD was slower than the initial phase. The divergence from a single exponential decline was most likely due to an additional long latency slow depolarization. This is more apparent in a subsequent section on the response to multiple stimuli. The duration of TDs (6–12 mV amplitude) was measured from 138 cells ($n = 43$ vessels) and is shown in Figure 1. The distribution of TD durations is skewed to the left with a median duration of 2 seconds and a range of 1–28 seconds. The variation in duration was largely due to differences in the declining phase of the TD; thus, the time to peak for these same 138 cells did not vary to the same extent and averaged 96 ± 37 msec (see inset, Figure 1).

**Stimulus response relation.** Larger stimulus voltages gave rise to larger TDs. The voltage-response relation measured in 0.15-V increments was a smooth, steadily rising function as opposed to a stepwise relation. At a threshold membrane potential of approximately −48 ± 4 mV, a discontinuity was usually apparent in the voltage-response relation. TDs above threshold gave rise to a smooth muscle action potential (Figure 2; $n = 8$). Contraction could sometimes be visualized associated with the action potential. The action potential was not an all-or-none event; rather, the amplitude increased with stimulus voltage. The mechanism for these graded responses may be related to the nonuniform way in which the vessel is activated as shown in the following section on responses with distance from the stimulating electrodes. The maximum amplitude of the action potential was 47 ± 5 mV (20 cells, 8 vessels) and did not overshoot zero. As the amplitude of the action potential increased, the time to the peak of the action potential decreased. After the initial rapid depolarization and partial repolarization of the action potential, there was a secondary slow depolarization (Figure 2A). The amplitude of this secondary slow depolarization was dependent upon the intensity of the stimulus up to a maximum amplitude of 21 mV. The time required for repolarization to resting membrane potential from the peak of the action potential averaged 77 ± 33 seconds (20 cells, 8 vessels). For stimulus parameters that initiated TDs, slow depolarization occurred but was never associated with a second peak as seen during the action potential. The extent of slow depolarization was estimated by measuring the amplitude of responses at long times (i.e., 4 seconds and 20 seconds) with increasing stimulus voltage. When slow depolarization was estimated in this way, we observed that the threshold stimulus voltage for initiating a slow depolarization was greater than for generation of a TD. Furthermore, slow depolarization appeared at 4 seconds with lower stimulus strengths than at 20 seconds; that is, the duration of slow depolarization increased with increasing stimulus strength. Although the amplitude of the TD at which slow depolarization appeared differed between cells, all cells exhibited some slow depolarization if the stimulus strength was increased sufficiently. The amplitude of slow depolarization increased with stimulus strength in a manner analogous to the TD.

**Comparison of strength-duration relation in coronary and mesenteric arteries.** To determine how the stimulus parameters necessary to evoke a TD in the coronary vasculature compare with those necessary to elicit an EJP, the response to TMS in the coronary artery was compared with the response in similar-sized segments of the inferior mesenteric artery. The same stimulating electrodes and configuration for stimulating transmurally (i.e., distance from the vessel and position of recording electrode) were used for the two vessels. The strength-duration relation for each vessel

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**Figure 1.** Distribution of transient depolarization (TD) durations in the guinea pig coronary artery. TDs were recorded in response to single stimuli in 138 cells from 43 vessels. Inset: Semilogarithmic plot (base 10) of the decline of two TDs from different preparations. Both TDs had a similar amplitude and time to peak but differed in the declining phase. The briefer-duration TD (1.2 seconds) follows an exponential decline up to 700 msec. The longer-duration TD (8.5 seconds) follows the same falling exponential for the first 300 msec; thereafter, the rate of repolarization is slower. Note that $\tau_f = 1/[(\ln 10)log y_2 - log y_1]$, where $y_1$ and $y_2$ are the amplitudes of the TD at which slow depolarization appeared. This formula gives a good fit for the TD at the lower stimulus strength ($\tau_f = 450 ± 139$ msec).
Changes in membrane potential in a pig coronary artery. A: Stimulus voltage-response relation in the guinea pig coronary artery. A: Changes in membrane potential in a single cell associated with three different stimulus voltages (9.5, 11, and 13 V). A transient depolarization (TD) is obtained with the smaller stimulus voltage and action potentials with the larger stimulus voltages. The action potential contains both a rapid initial spike and a slower rising and declining phase. Note that with a larger stimulus the time to peak of the action potential is less and the amplitude is larger. A stimulus artifact precedes all three electrical events. B: Plot of the voltage-response relation in another cell. Stimulus intensity was increased in 0.15-V increments. The left ordinate refers to the peak amplitude of the TD or action potential. The right ordinate refers to the corresponding membrane potential at the peak of the response. The threshold for action potential initiation was —50 mV.

was then measured by determining the voltage and duration of stimulus pulse necessary to elicit a 6.6-mV EJP (nonfacilitated). There was no significant difference between the strength-duration relation in the coronary artery (n = 6) and the mesenteric artery (n = 4) (Figure 3). However, the time course of the electrical events in these two preparations differed in that the EJP in the mesenteric artery (time to peak, 51 ± 14 msec; \( \tau_r \), 141 ± 37 msec) was more rapid than the TD in the coronary artery (time to peak, 97 ± 30 msec; \( \tau_r \), 450 ± 139 msec). No other measurements were made in mesenteric vessels in the remainder of this study.

Responses with distance. Thus far, we have presented results obtained by recording intracellularly near (± 250 µm) the stimulating electrodes in the coronary artery. Random impalement of cells through the adventitia within this portion of the preparation gave rise to TDs that exhibited very similar stimulus-response relations. To determine to what degree these responses are representative of the tissue as a whole, responses were also measured from cells on the intimal side of the media as well as at distance from the stimulating electrodes. TDs and action potentials could be recorded from smooth muscle cells located on the intimal side of the media (n = 2), although they required somewhat larger voltages (0.05-msec duration) due to the less effective delivery of current. To record responses at distance from the site of stimulation, cells were impaled through the adventitia near the stimulating electrodes and at 1-, 2-, and 3-mm distance from them. A stimulus voltage was selected that gave rise to an action potential near the stimulating electrodes. At 1-mm distance, the amplitude of the response was significantly less and the rate of depolarization was significantly slower. At 2-mm distance, the response was slower and smaller than at 1-mm distance. Beyond 3 mm, responses could not be recorded (Figure 4, n = 3).

Effect of depolarization with potassium on TD. Experiments were undertaken with potassium to determine whether the TD amplitude was related to the membrane potential (E_m) in the coronary artery. With increasing concentrations of \([K]_o\) (Na + substitution), the membrane depolarized (maximum slope = 49 mV/decade) and the amplitude of the TD decreased. Above 34.8 mM \([K]_o\) (E_m = —35 mV), the TD was abolished (Figure 5, n = 5). At 17.4 mM \([K]_o\) (E_m = —50.5 mV), the amplitude of the TD was reduced to 65% of control; however, the time course of the TD was not changed (time to peak: control, 85 ± 34 msec; 17.4 mM \([K]_o\), 100 ± 53 msec; \( \tau_r \); control, 423 ± 120 msec; 17.4 mM \([K]_o\), 449 ± 191 msec). It is of interest to compare these results with those presented for ACh in a subsequent section where reduction of the TD to 65% of control amplitude is associated with large changes in the time course of the TD.

Effect of tetrodotoxin, guanethidine, low calcium solution, and cold storage. These experiments were undertaken to determine whether the responses we observed were neural in origin. During a 20-minute exposure to TTX (10^-7 M), the amplitude of a TD obtained with a constant stimulus voltage was unchanged (control, 6.9 ± 1.9 mV; TTX, 6.5 ± 1.5 mV, n = 5), and action potentials could still be generated by increasing the amplitude of the stimulus voltage.

Guanethidine has been reported to eliminate the EJP in a number of different blood vessels.\textsuperscript{14,26,30} Since the effects of guanethidine are stimulus-dependent,\textsuperscript{21,22} guanethidine (2 × 10^-5 M) was tested by applying it to the tissue for 45 minutes while repetitively stimulating at 0.5 Hz (n = 5). The voltage-response relation was then measured and compared with responses measured during the control period. Following repetitive stimulation for 45 minutes, there was usually a small shift in the voltage-response relation. However, a similar shift was observed by repetitively stimulating vessel segments for 45 minutes in the absence of guanethidine (n = 3). This indicated that the shift was the result of repetitive stimulation, not guanethidine. No specific effect could be ascribed to guanethidine.

Coronary arteries from three guinea pigs were cold...
stored at 2°C for 4 days to denervate them. The resting membrane potential in these vessels was \(-47 \pm 2.5\) mV (n = 3, 14 cells). Stimulus voltages between 7 and 12 V (0.05-msec duration) consistently elicited TDs in fresh vessels. This same range of voltages did not give rise to TDs in cold-stored vessels. In addition, whereas contraction was observed in fresh vessels stimulated with 12–15 V (0.05-msec duration), contraction could not be elicited in cold-stored vessels within this same voltage range. Contraction occurred in cold-stored vessels with long-duration (1–2 msec) pulses.

Reduction of extracellular calcium ([Ca\(^{2+}\)]) reduces the amplitude of EJPs presumably by reducing transmitter release. To determine whether the TD in the guinea pig coronary artery was dependent upon [Ca\(^{2+}\)], experiments were performed in low calcium solution in the presence or absence of the inorganic calcium channel antagonist cadmium. TDs were initiated at 0.05 Hz before and during superfusion with low calcium (0.25 mM) solution to which 2.25 mM CdCl\(_2\) had been added (n = 4). The TD was reduced to 33 \(\pm\) 9% of control after 7–13 minutes superfusion with this low Ca\(^{2+}\)/Cd\(^{2+}\) solution, and the membrane depolarized from \(-61.6 \pm 3\) mV to \(-45.6 \pm 5.2\) mV. Fifteen to 27 minutes after returning to 2.5 mM Ca\(^{2+}\)-containing solution, membrane potential had returned to the control level, but the TD was only 23 \(\pm\) 16% of control amplitude. A stimulus voltage-response amplitude relation measured at this time indicated a reduction in amplitude of the TD at all voltages tested. In Figure 6, an example of the effects of cadmium on TD amplitude are shown.

In contrast, in the absence of cadmium, reduction of calcium to either 0.25 mM or 0 mM for 6–10 minutes led to depolarization from \(-60.7 \pm 3.4\) mV to \(-41.7 \pm 7.6\) mV (n = 6). In 0.25 mM Ca\(^{2+}\), the TD was reduced to 47 \(\pm\) 16% of the control amplitude (n = 3), whereas in 0 mM Ca\(^{2+}\), the TD was reduced to 21 \(\pm\) 6% of control (n = 3). Return of calcium under both circumstances led to immediate repolarization and return of the TD to control amplitude.

**Effect of adrenergic antagonists and an agonist.** In most arterial preparations, the TD is not blocked by \(\alpha\)-antagonists. To determine whether this is also the case for the TD in the coronary artery, we tested the \(\alpha\)-adrenergic antagonists phentolamine (10\(^{-5}\) M; n = 4) and prazosin (10\(^{-6}\) M; n = 4). The effects of these antagonists on the TD were tested by stimulating transmurally at 0.05 Hz before and during introduction of the drug. During a 15-minute exposure, neither the
amplitude of the TD obtained with a given voltage nor the action potential amplitude was reduced.

To determine whether the TD might be due to the action of NE on a receptor not blocked by α-antagonists, the response to a high concentration of NE (10 mM) was tested. This concentration represents the upper limit predicted to be present in a narrow junction during the first instant of release and should be sufficient to occupy all available postjunctional receptors. Seven vessel segments were tested. In five vessels, NE produced a depolarization of 14.8 ± 4.5 mV, which diminished with time but did not entirely return to control after 5 minutes (n = 5) or 20 minutes (n = 3). In the other two vessels, NE hyperpolarized the cells by 6 and 8 mV. In one of the vessels that hyperpolarized, propranolol (0.1 mM) abolished a 6-mV hyperpolarization produced by 0.1 mM NE. Perivascular nerves were stimulated at 0.05 Hz before and during exposure to 10 mM NE. In the vessels that depolarized with NE, the TD was reduced in amplitude, and in those that hyperpolarized, the amplitude of the TD increased. To test whether 10 mM NE is a maximal concentration of this agonist, NE concentration was raised from 10 mM to 20 mM after 20 minutes exposure to 10 mM NE (n = 2). There was no further depolarization when NE concentration was increased in this manner. In all vessels, larger amplitude TDs and action potentials could still be initiated with larger stimulus voltages (0.05-msec duration) in the presence of either 10 or 20 mM NE. In four vessel segments, 0.01 mM NE produced no depolarization and no change in the amplitude of the TD.

It has been suggested that ATP may be responsible for the EJP, thus explaining the lack of effects of α-receptor antagonists. Support for this hypothesis has included selective blockade of the EJP with the purinergic antagonist arylazido aminopropionyl adenosine triphosphate and desensitization with the ATP analogue α,β-methylene ATP. Coronary segments were exposed to 10⁻⁶ M or 2 × 10⁻⁵ M α,β-methylene ATP for 16-50 minutes while stimulating transmurally at 0.05 Hz. Introduction of 10⁻⁶ M α,β-methylene ATP produced small transient changes in membrane potential; however, after 16 minutes of exposure to this ATP analogue, the membrane potential and the amplitude of the TD were not significantly different from control (control membrane potential, −62.9 ± 5 mV; α,β-methylene ATP membrane potential, −62.2 ± 5.4 mV; TD amplitude, 95.8 ± 8% of control; n = 6). Membrane potential and the TD amplitude were also not significantly different after 6 or 20 minutes of exposure to 2 × 10⁻⁵ M α,β-methylene ATP (n = 4), and in one vessel, the TD amplitude was not reduced after 50 minutes of exposure to 2 × 10⁻⁵ M α,β-methylene ATP (Figure 7).

Response to Multiple Stimuli

Vessel segments from five animals were repetitively stimulated for 1 minute at frequencies between 0.05 Hz and during exposure to 10 mM NE. In the vessels that depolarized with NE, the TD was reduced in amplitude, and in those that hyperpolarized, the amplitude of the TD increased. To test whether 10 mM NE is a maximal concentration of this agonist, NE concentration was raised from 10 mM to 20 mM after 20 minutes exposure to 10 mM NE (n = 2). There was no further depolarization when NE concentration was increased in this manner. In all vessels, larger amplitude TDs and action potentials could still be initiated with larger stimulus voltages (0.05-msec duration) in the presence of either 10 or 20 mM NE. In four vessel segments, 0.01 mM NE produced no depolarization and no change in the amplitude of the TD.

FIGURE 5. Comparison of the effect of various concentrations of extracellular potassium concentration on membrane potential (dotted line) and the amplitude of the transient depolarization (TD) (solid line). With increasing concentrations of potassium, the membrane depolarizes, and the amplitude of the TD is diminished. When the membrane is depolarized above −35 mV, the TD is abolished. (Data shown are x ± SD, n = 5 vessels, 18 cells).

FIGURE 6. Effect of low-calcium solution (0.25 mM) with 2.25 CdCl₂ on membrane potential and the amplitude of the transient depolarization (TD). Upper trace: Intracellular recording with time in a single cell before, during, and after superfusion with low Ca²⁺/Cd²⁺ solution. In the presence of low Ca²⁺/Cd²⁺ solution, the membrane depolarizes 10 mV, and the TD is reduced from 6 mV to 2.1 mV (a stimulus artifact contaminates the smallest amplitude responses). When low Ca²⁺/Cd²⁺ solution is replaced with regular calcium-containing solution, the membrane slowly repolarizes to control potential but the TD does not return to control amplitude. Lower graph: Plot of the stimulus voltage–response amplitude relation for a single cell before exposure to low Ca²⁺/Cd²⁺ solution (solid line) and after the membrane has returned to the control level following washout of low Ca²⁺/Cd²⁺ solution (dotted line). Note that responses to transmural stimulation have been suppressed at all voltages tested following exposure to cadmium.
and 2 Hz. A stimulus strength that gave rise to an 8–10 mV TD was used. No cells exhibited slow depolarization at a stimulus frequency of 0.05 Hz. Two of five vessels slowly depolarized by 1 and 4 mV at 0.1 Hz and required 42 and 48 seconds, respectively, for repolarization to be complete (measured 1.5 seconds after initiating the final EJP). The mean maximum slow depolarization at 0.25, 0.5, 1, and 2 Hz was 5.4 ± 3.0 mV, 8.8 ± 4.7 mV, 12.1 ± 2.8 mV, and 15.3 ± 2.5 mV, respectively. Repolarization following cessation of stimulation required 59 ± 12 seconds, 84 ± 14 seconds, 94 ± 23 seconds, and 107 ± 32 seconds, respectively (Figure 8). At 2 Hz, summation sometimes led to a single action potential at a threshold membrane potential of −47 ± 3 mV. An action potential was consistently elicited when nerves were stimulated at frequencies greater than 2 Hz.

TTX (10−4 M), guanethidine (2 × 10−5 M), phenolamine (10−5 M), prazosin (10−6 M), and 10 mM NE were also tested on the slow depolarization. All of these substances were ineffective in eliminating the slow depolarization.

Effect of a Cholinergic Agonist and Antagonist

To determine whether TDs may be related to cholinergic nerves, we investigated the effects of atropine (10−5 M). Perivascular nerves were stimulated at 0.05 Hz at a constant voltage before and during a 15-minute exposure to atropine. Atropine had no effect on either the amplitude or time course of the TD. In addition, atropine did not affect the slow depolarization and repolarization associated with repetitive stimulation or action potentials (n = 5).

The experiments with atropine suggest that muscarinic receptors are not involved in generation of the TD. However, muscarinic receptors are present in the guinea pig coronary artery since ACh induces an atropine sensitive hyperpolarization.40 It was suggested in these studies that membrane hyperpolarization was due to an increase in potassium conductance because the response was not altered in low Na+ or Cl− solution and a reversal potential equivalent to EK could be demonstrated. In the present study, we investigated the effect of ACh on generation of the TD. The vessel was stimulated transmurally with a constant voltage at 0.05 Hz before and during exposure to ACh. Cells significantly hyperpolarized with ACh from −62.7 ± 2.5 mV in control Krebs solution to −76.2 ± 7.0 mV (n = 4). In Figure 9, the effects of ACh on the TD were a reduction in τr, with 10−5 M ACh, and a reversal potential equivalent to EK could be demonstrated. The first significant effect of ACh on the TD was a reduction in τr, with 10−5 M ACh. In 10−5 M ACh, the TD amplitude decreased from 9 ± 1.7 mV to 5.5 ± 1.3 mV, τr decreased from 478 ± 140 msec to 134 ± 37 msec in 10−5 M ACh, and the time to peak decreased from 80 ± 19 msec in the control to 38 ± 4 msec in 10−5 M ACh (Figure 9, n = 7). The effects of ACh were blocked with 10−5 M atropine (n = 4).

The effects of ACh on the TD may be due to changes in EK, GK, or both. To further explore the effects of hyperpolarization on the TD, we have compared the effects of hyperpolarization with ACh to those obtained by exposing the tissue to potassium-free solution for 30 minutes followed by potassium repletion. When potassium was removed from solution, slow membrane fluctuations were sometimes observed but not constant depolarization. Upon return of potassium after 30 minutes, cells hyperpolarized from −59.5 ± 3.8 mV to −68.2 ± 7.0 mV (n = 4). In Figure 10, the effects of ACh and potassium repletion on membrane potential and the TD are compared in two cells. The amplitude of the TD during membrane hyperpolarization was increased to 160 ± 16% of control following potassium repletion, but the time course of the TD was the same as that obtained from an equal amplitude TD in control solution.

Discussion

Transmural stimulation of the guinea pig coronary artery with brief duration (0.05 msec) stimuli leads to...
FIGURE 8. Effect of stimulus frequency on the level of slow depolarization in a single cell in the coronary artery (MP = -66 mV). At 0.25 Hz and at higher frequencies the TDs begin to summate and the membrane depolarizes during the stimulus. After the end of stimulation the membrane repolarizes with a time course dependent upon the previous frequency of stimulation. At frequencies that do not produce summation there is no facilitation of TD amplitude. Stimulus artifacts appear as downward deflections on the recordings.

TDs, action potentials, and slow depolarization. In this way, the electrical events in the coronary artery superficially resemble responses recorded from a variety of other blood vessels including the tail artery, mucosal arterioles, saphenous artery, mesenteric artery, and basilar artery. However, the electrical events recorded in the coronary artery are unique from those in other systemic arteries in that they are partially resistant to the removal of calcium and are not blocked in guanethidine, TTX, or α,β-methylene ATP (O.D. Hottenstein et al, unpublished observations). Slow depolarization in the coronary artery is also unique in that it is not blocked by α-receptor antagonists or guanethidine.

The electrical activity recorded in the isolated coronary artery may be due to one of three possible mechanisms: stimulation of perivascular nerves resulting in release of neurotransmitter substances, direct stimulation of the vascular smooth muscle cells, or stimulation of a third cell type in the blood vessel that is coupled either directly or indirectly to the smooth muscle. The evidence in favor of each of these three hypotheses is discussed below.

Evidence for the neural origin of the responses includes the following observations. First, TDs were no longer obtained after 4 days of cold storage at 2°C, a procedure that causes deterioration of nerves. Second, the stimulus durations at which TDs could be elicited (0.01–0.5 msec) were equivalent to those necessary to elicit equal amplitude EJPs in the guinea pig mesenteric artery. Nerve-evoked EJPs have previously been demonstrated in the mesenteric artery. EJPs elicited in the guinea pig inferior mesenteric artery with the same transmural stimulation are sensitive to TTX (10^-6 M) (O.D. Hottenstein et al, unpublished observations).

The third observation in keeping with the hypothesis that TDs are neural in origin is that exposure of the vessel to a low calcium solution with cadmium led to a significant reduction in the amplitude of the TD, not only during the period of depolarization but also subsequently when membrane potential was restored to the control level. Cadmium is a potent calcium channel antagonist. Both adrenergic and cholinergic transmission are effectively blocked by cadmium. The actions of cadmium are known to persist following washout with a time course dependent upon the concentration tested and the period of exposure. For example, the cholinergic end plate potential is abolished for 40 minutes following washout of 100 μM cadmium and requires 70–80 minutes to return to control amplitude. Forshaw has suggested that these long-term effects of cadmium are due to the high affinity of cadmium for membrane constituents containing sulphydryl groups.

Although the TD was reduced in amplitude in calcium-free solution, it was not abolished. It may be that a more complete removal of extracellular calcium is required for complete abolition of the TD. For

FIGURE 9. Relation of transient depolarization (TD) time course to ACh concentration. Points are mean ± SD (n = 7 vessels, 25 cells). *Significantly different from values measured in the absence of acetylcholine. "Decay constant" refers to τ values. The decrease in TD amplitude is accompanied by decreases in the time to peak and decay time constant.
instance, Glass et al\textsuperscript{40} and Baron et al\textsuperscript{47} showed that, whereas the release of substance P was blocked in calcium-free solution with 0.1 mM EGTA, blockade of methionine-enkephalin required 1 mM EGTA.

The time course of TDs recorded in the coronary artery are very similar to EJPs recorded in other blood vessels. This is apparent in comparing the two examples of Figure 3. The slow depolarization observed in the coronary artery is also similar to that reported in the rat tail artery with perivascular nerve stimulation.\textsuperscript{24} These observations are not direct evidence for a neural mechanism, but they do suggest that the time course of events responsible for the TD must be similar to those necessary to elicit an EJP.

Further parallels between the electrical events in the coronary artery and those evoked with perivascular nerve stimulation in other vessels include the lack of sensitivity to α-receptor antagonists,\textsuperscript{11,12,14,15,16,26,27} the modification in time course of TDs by ACh,\textsuperscript{12} and the alteration in amplitude of the TD with the level of membrane potential.\textsuperscript{48}

Perhaps the most serious difference between the electrical events in the coronary artery and those of other arteries is the observation that responses are not blocked by TTX, whereas in many other arteries it has been shown that EJPs are suppressed by TTX\textsuperscript{12,18,19,20,33} (O.D. Hottenstein et al, unpublished observations). However, it is known that axonal action potentials are not essential for neurotransmitter release and that any sufficiently large depolarization will release transmitters in the presence of TTX.\textsuperscript{49} For instance, a TTX-insensitive release of substance P from myenteric neurons has been reported.\textsuperscript{48} Since the responses we recorded were near the stimulating electrodes, it is possible that individual varicosities in the field of stimulation released transmitters by direct depolarization. In the guinea pig ear artery, guanethidine-sensitive EJPs have been demonstrated in the presence of TTX and direct release from varicosities was suggested.\textsuperscript{17}

The electrical events recorded in the coronary artery could be obtained with very brief duration stimuli (≥ 0.01 msec). Furthermore, TDs began with a latency of up to 20 msec from the onset of stimulation. These characteristics are not readily compatible with direct stimulation of the smooth muscle since the time constant of vascular smooth muscle is relatively long (e.g., in the pig and dog coronary artery values from 290 to 410 msec have been reported\textsuperscript{20,35}) and since by definition direct stimulation of the smooth muscle membrane requires that it be depolarized during the stimulus and not after the stimulus has ceased. The possibility still exists, however, that electrical stimulation of the smooth muscle cell initiates some other event within the cell, which in turn leads to membrane depolarization, but the mechanism for such a sequence of events is at present obscure.

In support of the third hypothesis (i.e., contribution from a third cellular component to generation of the TD), is a brief report made by Nagao and Suzuki\textsuperscript{52} on
the rabbit basilar artery. In this vessel, similar TTX-resistant TDs were shown to be dependent on the integrity of the endothelium; thus, it was suggested that the smooth muscle is activated by way of the endothelium. It remains to be determined if such a relation exists in the guinea pig coronary artery.

Evidence from the present study for involvement of a third component in generation of TDs is more inferential: if direct muscle stimulation is discounted, then the lack of effect of drugs that typically inhibit EJPs in other blood vessels (TTX, guanethidine, and α,β-methylene ATP) increases the likelihood that a nonneural mechanism may be involved.

ACh was shown in the guinea pig coronary artery to have marked effects on both resting membrane potential and the time course of the TD. The actions of ACh were muscarinic since they were blocked by atropine. The effects of ACh on membrane potential and the response to transmural stimulation are similar to those reported by others. In a number of vascular preparations, it has been shown that the inhibitory actions of ACh are dependent on an intact endothelium. We have not studied the endothelial dependence of the effects of ACh in the guinea pig coronary artery, although our histological studies indicate that the endothelium is intact.

From the present available data, it is not possible to confidently attribute the events we recorded to any one of the three hypothetical mechanisms of activation. The two most likely mechanisms, however, are release of a neurotransmitter substance other than NE, ATP, or ACh or alternatively endothelium-dependent activation of the smooth muscle. Regardless of the mechanism, the fact that these events can give rise to contraction suggests that they represent a potentially important mechanism for generation of tone in the coronary artery.

It is of interest that the responses in the coronary artery most closely resemble those of the basilar artery. The similarities between these two vessels include the observation that both vessels exhibit a prominent "tail" component to the TD. Furthermore, both vessels require extremely large concentrations of NE to evoke depolarization. It is suggested, in fact, that α-receptors are absent in the rat basilar artery. Like the coronary artery, TDs in the basilar artery are not blocked by TTX or guanethidine, and thus, it has been concluded that the responses are either nonadrenergic or nonneural. In a recent study by Surprenant et al. of transmural stimulation in the rat basilar artery, it was concluded that the responses were nonneuronal since, besides TTX and guanethidine, they were not blocked by low calcium solution.

In conclusion, we have described electrical events occurring in the coronary artery with stimulus parameters that traditionally activate peripheral nerves. The responses resemble those observed in other blood vessels with perivascular nerve stimulation but also exhibit certain fundamental differences. Because of these differences, it is not possible to directly associate the activity we have recorded with any of the contractile studies discussed in the introduction. Regardless of whether the responses are due to stimulation of nerves, muscle, or a third cellular component, they represent a novel mechanism of vascular activation.

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