Desensitization of Chick Embryo Ventricle to the Physiological and Biochemical Effects of Isoproterenol

Evidence for Uncoupling of the $\beta$ Receptor-Adenylate Cyclase Complex

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SUMMARY To determine if cardiac tissue is capable of modulating its response to a stimulating hormone, we studied desensitization to the positive inotropic effect of catecholamines on embryonic chick ventricular tissue using a phase contrast microscope-video motion detector system and correlated the contractility findings with concurrent observations of $\beta$-adrenergic receptor properties and adenylate cyclase activity. Incubation for 30 minutes with 1 $\mu M$ isoproterenol produced a diminution in the subsequent inotropic response to 0.1 $\mu M$ isoproterenol to 35 ± 8% (mean ± SEM) of the initial response. This desensitization to the positive inotropic effect of isoproterenol was catecholamine-specific and was not accompanied by alteration in the inotropic response to Ca$^{2+}$. To investigate the mechanism of desensitization, we studied properties of the $\beta$-adrenergic receptor in homogenates of chick embryo ventricle using [$^3$H]dihydroalprenolol as a ligand. Thirty minutes of incubation with 1 $\mu M$ isoproterenol produced no change in $\beta$-adrenergic receptor density (92.8 ± 5.1 fmol/mg protein) and only a small change in receptor affinity ($K_D = 5.2 \pm 0.3$ nM vs. 7.0 ± 0.3 nM; $P < 0.01$). Receptor affinity for isoproterenol, as judged by [$^3$H]dihydroalprenolol displacement, was not changed significantly. The adenylate cyclase stimulation by isoproterenol in similarly prepared tissue, however, was reduced to 29% of the control value after 30 minutes of exposure to 1 $\mu M$ isoproterenol. Adenylate cyclase sensitivity was restored by guanosine 5'-($\beta$,\gamma-imino)triphosphate. Thus, desensitization of physiological responsiveness of ventricular tissue to a $\beta$-adrenergic agonist was accompanied by little change in $\beta$-adrenergic receptor properties but by marked diminution in adenylate cyclase responsiveness. These observations suggest that uncoupling of the $\beta$-adrenergic receptor-adenylate cyclase complex may be the mechanism of short-term desensitization of ventricular muscle to the positive inotropic effects of isoproterenol. Circ Res 47: 493-501, 1980

AN IMPORTANT effect of catecholamines is to increase the force of contraction of the myocardium. This response of the heart to $\beta$-adrenergic stimulation is a homeostatic mechanism operative in physiological circumstances, such as exercise, and in pathophysiological circumstances, such as congestive heart failure. Diminution in the responsiveness of the heart to catecholamines would be expected to have potentially important clinical manifestations. Clinically, it has been long suspected that tachyphylaxis to the cardiac inotropic effects of catecholamines does occur (Goldstein et al., 1974). The nature and mechanism of this phenomenon have, however, not been fully elucidated in a suitable model in vitro or in vivo.

Other investigators, in studies of rat (Harden et al., 1976; Tsai and Chen, 1978; Williams et al., 1977) and canine (Alexander et al., 1975) myocardium, have demonstrated the existence of specific $\beta$-adrenergic receptors that are linked to adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1], as is the case in a number of other hormone-receptor systems (Neer, 1973; Sayers et al., 1972; Mendelson et al., 1975). Available evidence suggests that $\beta$-adrenergic agonists elicit a physiological response from some tissues by a sequence of events including hormone binding to a cell surface receptor that is coupled to adenylate cyclase. The coupling may be modulated by a number of factors, including guanine nucleotides. Adenylate cyclase is activated and increases the intracellular cyclic adenosine monophosphate (cAMP) concentration. (Alexander et al., 1975; Tsai and Chen, 1978). This in turn...
activates a protein kinase that phosphorylates a regulatory protein (or proteins) that ultimately regulates the physiological activity of the cell (Kuo and Greengard, 1969).

In 1950, Barry demonstrated that, during continuous exposure to epinephrine, its chronotropic effect on embryonic chick heart wanes. Desensitization to the biochemical effects of hormone stimulation, including catecholamine exposure, occurs in a number of systems (Mickey et al., 1975; Galper and Greengard, 1969).

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Desensitization to \( \beta \)-adrenergic agonists has been accompanied by a decrease in the apparent number of \( \beta \)-adrenergic receptors in some systems (Mukherjee et al., 1975) or with “uncoupling” of the \( \beta \)-adrenergic receptor-adenylate cyclase complex in others (Harden et al., 1979). There are, to our knowledge, no data available concerning mechanisms by which myocardial responses to a relatively brief exposure to \( \beta \)-adrenergic agonists may be regulated. As an approach to this problem, we have evaluated the biochemical and physiological responses of embryonic chick ventricular myocardium to the \( \beta \)-adrenergic agonist isoproterenol. Our findings demonstrate partial desensitization of the inotropic response to isoproterenol and define an alteration in the coupling of the \( \beta \)-adrenergic receptor-adenylate cyclase complex that may account for the altered physiological response.

**Methods**

**Chick Embryo Ventricular Tissue**

Embryos were removed from 10-day-old Legg-Whitehorn chick eggs (Spafas, Inc.). Hearts were taken from the embryos and trimmed free of great vessels and atria while immersed in oxygenated physiologic salt solution. A 0.5- x 1.0-mm strip of ventricular tissue was mounted with 6-0 silk thread on the tip of a glass capillary through which was threaded a fine silver stimulating electrode. The capillary with attached ventricular tissue was mounted in a modified Sykes-Moore chamber with inlet and outlet ports (Belco Glass, Inc.). The chamber was connected by polyethylene tubing to four syringe pumps (Harvard Instruments) so that the tissue could be superfused sequentially with separate test solutions. Flow characteristics of the chamber were determined by indocyanine green dye washout. Solution bathing the ventricular tissue mounted in the center of the chamber could be changed with a time constant of 15 seconds at a flow rate of 0.96 ml/min.

The optical apparatus was supported on an air table to dampen building vibrations. A portion of the ventricular tissue was magnified with a 10× objective, and the image monitored by a low light level silicon television camera (Dage 650) attached to the microscope observation tube using a 2× coupler. The television camera video output was connected to a video motion detector (Colorado Video 633) and displayed on a Conrac television monitor. The total magnification of the image on the monitor screen was 500×. The television camera had an interlace defeat to improve frequency response so that the image was composed of 262 raster lines. The motion detector monitored a selected raster line segment and provided analog position data every 16 msec for an image border of the ventricular tissue moving along the raster line. The analog voltage output from the motion detector was filtered at 15 Hz with a 48 dB/octave low-pass active filter and calibrated to indicate actual \( \mu \)m of motion. The first derivative of the position signal was obtained electronically and was recorded as velocity of motion in \( \mu \)m/sec. These analog signals were recorded using either a Hewlett-Packard 4560 optical recorder or a Brush 2200 recorder.

The ventricular tissue was stimulated to contract at 100/min throughout all experiments. The magnitude of shortening did not depend on stimulus strength in the range used in these experiments. Stimulus strength was constant for a given experiment and was independent of the experimental conditions employed. Experiments in which rate of spontaneous contraction exceeded 100/min were discarded. When ventricular segments were superfused with physiologic salt solution containing 0.9 mM Ca\(^{2+}\), the mean amplitude of motion during contraction was about 10 \( \mu \)m. The same segment of solutions were stable for at least 8 hours as judged by reproducible inotropic responses and lack of discoloration. Fresh physiologic salt solution and isoproterenol solutions were prepared daily. Solutions with high calcium concentration (5.4 mM) used in some experiments were otherwise identical to the physiologic salt solution. All solutions were gassed with 95% O\(_2\)-5% CO\(_2\) and were at pH 7.4.

**Measurement of Contractility**

The Sykes-Moore chamber containing the ventricular tissue was placed on the stage of an inverted phase contrast microscope (Leitz Diavert), which was entirely enclosed in a Lucite box, the contents of which were maintained at 37 ± 0.5°C. The inlet to the Sykes-Moore chamber was connected by polyethylene tubing to four syringe pumps (Harvard Instruments) so that the tissue could be superfused sequentially with separate test solutions. Flow characteristics of the chamber were determined by indocyanine green dye washout. Solution bathing the ventricular tissue mounted in the center of the chamber could be changed with a time constant of 15 seconds at a flow rate of 0.96 ml/min.

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ventricular tissue was monitored throughout each experimental study, and changes in recorded amplitude and velocity of motion were used as an index of change in contractility. Preparations were stable for at least 3 hours under the experimental conditions described.

Measurements of contractility of ventricular strips from embryonic chick heart recorded by this optical-video method are comparable qualitatively to records obtained from the same tissue using an isometric force transducer technique, both in time course of response and relative magnitude of response (Barry et al., 1975). Embryonic chick ventricular tissue has very little fibrous tissue to impede diffusion of solutes and thus has favorable properties for temporal resolution of inotropic events. However, the lack of fibrous tissue makes the samples fragile. It is quite difficult to record stable signals from this tissue mounted in a force transducer, and maximal inotropic stimulation frequently causes the tissue to pull apart.

Protocol

To determine the contractile response to isoproterenol, the following procedure was performed. A regularly contracting ventricular strip was superfused for 30 minutes with 0.9 mM Ca\(^{2+}\) physiologic salt solution, and a stable baseline of contractility was established. The tissue then was superfused with 0.1 \(\mu\)M (-)isoproterenol for 5 minutes, and the inotropic response was recorded. The contractile response reached a plateau by 3 minutes in all experiments. The tissue then was superfused with 1 \(\mu\)M isoproterenol for 30 minutes, followed by a 10-minute washout period with 0.9 mM Ca\(^{2+}\) physiologic salt solution to reestablish baseline contractility. There was a final 5-minute superfusion with 0.1 \(\mu\)M isoproterenol for comparison with the original inotropic response. Concentration-effect curves for isoproterenol were constructed by superfusing myocardial samples with progressively increasing concentrations of isoproterenol (10\(^{-10}\) to 10\(^{-6}\) M) for 3 minutes at each concentration.

To compare the contractile response to an elevation of superfusate [Ca\(^{2+}\)] to 5.4 mM, the same procedure was followed, except that the step involving exposure to 1 \(\mu\)M isoproterenol was replaced by exposure to 5.4 mM Ca\(^{2+}\). In a separate series of experiments, the ventricular samples were superfused with 0.1 \(\mu\)M isoproterenol as described above, but the tissue then was superfused with 5.4 mM Ca\(^{2+}\) for 30 minutes, rather than with 1 \(\mu\)M isoproterenol.

Receptor Binding Studies

Chick embryo ventricular tissue was prepared in physiologic salt solution containing 1 mM ascorbic acid, pH 7.4, as described for the contractility experiments. During preparation, the tissue was immersed continuously in solution gassed with 95% O\(_2\)-5% CO\(_2\). The tissue was washed in physiologic salt solution to remove blood and then incubated with gentle shaking at 37°C in physiologic salt solution, in the presence or absence of 1 \(\mu\)M isoproterenol. The tissue was not stimulated to contract during this period. At the end of the incubation, the pH of media remained 7.4. The tissue was washed with 5 ml physiologic salt solution four times to remove isoproterenol, then resuspended in assay buffer [75 mM Tris-Cl-25 mM MgCl\(_2\) (pH 7.4)], and homogenized using a Brinkmann Polytron homogenizer at 40% speed (four 10-second bursts) at 4°C. The resulting suspension was centrifuged at 50,000 \(g\) for 10 minutes, the supernatant was discarded, and the pellet was resuspended in assay buffer using the Polytron in identical fashion.

One hundred microliters of tissue suspension were added to 25 \(\mu\)l (-) [\(3H\)]dihydroalprenolol (\(3H\) DHA) (New England Nuclear; specific activity, 45 Ci/mmol) at varying concentrations, in the presence or absence of 2 \(\times\) 10\(^{-6}\) M (±) propranolol or assay buffer to make a final volume of 150 \(\mu\)l. The tubes were incubated with shaking at 37°C for 15 minutes, and the reaction was stopped by addition of 2 ml ice-cold medium. The reaction mixture was filtered through a glass fiber filter (Whatman GF/C) at reduced pressure, and the tube was washed three times with 5 ml ice-cold medium.

The filters were dried, 10 ml of Beckman Ready-Solv scintillation counting fluid were added, and the samples were counted at 45% efficiency in a Beckman liquid scintillation counter. The amount of nonspecific binding of radioactivity to filters was determined by performing the assay in the absence of tissue. Less than 0.1% of the total counts in the sample bound to the filter in the absence of tissue. Specific binding of \([3H]\)DHA at each concentration was defined as binding in the absence of 2 \(\times\) 10\(^{-6}\) M (±) propranolol minus binding in the presence of propranolol and was 50-75% of total counts bound, with greater specific binding at lower concentrations.

To assess the affinity of receptors for isoproterenol, the EC\(_{50}\) for isoproterenol displacement of \([3H]\)DHA was determined by performing the assay in the presence of a \([3H]\)DHA concentration near the K\(_D\) (5-7 nM) and a concentration of isoproterenol ranging from 10\(^{-7}\) to 10\(^{-4}\) M.

Adenylate Cyclase Assay

In order to correlate alterations in myocardial contractility responses to isoproterenol with alterations in adenylate cyclase responses to this \(\beta\)-adrenergic agonist, properties of adenylate cyclase were examined in ventricular tissue obtained and exposed to isoproterenol in a manner identical to that described for the contractility studies.

Adenylate cyclase activity was assayed by a modification of the method of Krishna et al. (1968) as described previously (Neer, 1973). The tissue was obtained as in the contractility studies, exposed to...
0.9 mM Ca2+ solution with or without 1 μM isoproterenol for 30 minutes, and then washed in 0.9 mM Ca2+ solution. Before assay, it was washed once more with 0.2 mM sucrose-10 mM MgCl2-1 mM dithiothreitol-1 mM EDTA-0.05 M Tris-Cl (pH 7.6). It then was homogenized in this buffer in a loose-fitting Dounce homogenizer and assayed without further treatment. Adenylate cyclase activity was measured using a 10-minute incubation at 37°C as described previously (Neer, 1973). The incubation mixture contained the following constituents according to the requirements of individual experiments: (+) propranolol (Sigma), 4 × 10⁻⁵ M; NaF, 8 mM; (-)-isoproterenol (Sigma), 2 × 10⁻⁵ M; guanosine 5’(β,γ-imino)triphosphate (Gpp(NH)p; ICN Pharmaceuticals, Inc.), 3 × 10⁻⁵ M; and (-)isoproterenol, 2 × 10⁻⁵ M, plus Gpp(NH)p, 3 × 10⁻⁵ M. The propranolol incubations were performed to assess the completeness of washing of the tissue after 30 minutes of exposure to 1 μM isoproterenol. In addition, the isoproterenol concentration-adenylate cyclase activity relationship was investigated by incubating tissue in 10⁻⁵ to 10⁻³ M isoproterenol concentrations in the absence of Gpp(NH)p. Protein determinations were by the method of Lowry et al. (1951) as modified by Bailey (1967).

Statistical Methods

Statistical analysis was performed on a Tektronix 4051 computer using Student’s paired t-test when a control and intervention study were performed on the same tissue and the unpaired t-test when different tissue preparations were compared (Snedecor and Cochran, 1967). Adenylate cyclase data was examined by analysis of variance.

Ligand binding data were analyzed by Scatchard analysis, using a least squares linear regression procedure. A logit transform was used to determine the EC₅₀ for the contractility concentration-effect curve.

Results

Contractile Response

The contractile response to exposure to 0.1 μM isoproterenol from a typical experiment is shown in Figure 1. The amplitude and velocity of contraction of the ventricular tissue while being superfused with 0.9 mM Ca²⁺ solution are shown (Control) followed by the response to 0.1 μM isoproterenol (Iso 1). There was a 42% increase in amplitude and a 100% increase in velocity of contraction. The tissue then was exposed to 1 μM isoproterenol for 30 minutes followed by superfusion with 0.9 mM Ca²⁺ solution and return to basal conditions for 5 minutes (Control). This resulted in a return to the baseline contractile state within 5 minutes. To see if there was desensitization to the physiological effect of isoproterenol, the tissue was superfused a second time with 0.1 μM isoproterenol-containing solution. This experiment demonstrates a diminished contraction amplitude and velocity response to 0.1 μM isoproterenol (Iso 2) compared with the response prior to incubation with 1 μM isoproterenol.

In six experiments, the initial exposure to 0.1 μM isoproterenol produced an increase to 183 ± 29% (mean ± SEM) of control amplitude. After 30 minutes of superfusion with medium containing 1 μM isoproterenol, the amplitude of contraction was 149% of control. Subsequent washout of isoproterenol reestablished the control amplitude of contraction, and rechallenge with 0.1 μM isoproterenol produced a diminished response to 131 ± 30% of control. Thus, when the ventricular tissue was stimulated with 0.1 μM isoproterenol before and after a 30-minute superfusion with 1 μM isoproterenol, the second response was diminished to 35 ± 8% of the initial response (P < 0.01).

To assess whether this decrease was specific for a β-adrenergic agonist or merely reflected deterioration of the tissue preparation or a nonspecific response to any inotropic stimulus, we exposed fresh tissue to 5.4 mM Ca²⁺ before and after 30 minutes of superfusion with 1 μM isoproterenol in six experiments. There was no decrease in the inotropic response of ventricular strips to Ca²⁺. The response to exposure to 0.1 μM isoproterenol before and after superfusion with 5.4 mM Ca²⁺ was also investigated. This concentration of Ca²⁺ produced a contractile response (294 ± 36% of control) similar in magnitude to that produced by 1 μM isoproterenol (308 ± 21% of control). The second response to
exposure to 0.1 μM isoproterenol after exposure to 5.4 μM Ca²⁺, however, was not significantly different from the response prior to exposure to 5.4 μM Ca²⁺ (112±29% of the initial response). This is in marked contrast to the attenuation of the response to 0.1 μM isoproterenol observed after exposure to 1 μM isoproterenol.

Concentration-effect relations for effects of isoproterenol on amplitude of contraction (expressed as the percentage of maximum amplitude) before and after 30 minutes of exposure to 1 μM isoproterenol are shown in Figure 2. The contractile response after 30 minutes of incubation with 1 μM isoproterenol was diminished at every concentration of isoproterenol greater than 1×10⁻¹⁰ M. However, the concentrations of isoproterenol required for half-maximal stimulation as judged from log-logit plots differed (1.3×10⁻⁶ vs. 4.4×10⁻⁸ M), consistent with a slight decrease in affinity of the receptor for agonist. There was no evidence for desensitization of the contractile effect of isoproterenol during the period the control concentration-effect curve was being determined.

β-Adrenergic Receptor Characteristics

To test the possibility that a decrease in β-adrenergic receptor number or affinity could account for the isoproterenol-induced desensitization, the receptor was assayed directly using the ligand [³H]DHA. Saturation curves were generated by adding increasing concentrations of [³H]DHA to a constant amount of particulate fraction protein. Scatchard analysis of these data gave linear plots (r > 0.90 in all cases) from which binding capacity and K_D were calculated. Figure 3 is representative of six sets of experiments. The mean density of β-adrenergic receptors in tissue not exposed to isoproterenol was 92.8 ± 5.1 fmol/mg protein, while the density in the isoproterenol-exposed tissue was similar at 96.1 ± 7.0 fmol/mg protein (P = 0.49). Thus, in contradistinction to the significant decrease in contractility, 30 minutes of exposure to 1 μM isoproterenol did not alter β-adrenergic receptor number as judged by [³H]DHA binding under these experimental conditions. The K_D for [³H]DHA in the control tissue was 5.2 ± 0.3 nm and was 7.0 ± 0.4 nm in isoproterenol-exposed tissue for six experiments. These dissociation constants differed significantly (P < 0.01).

The affinity of the β-adrenergic receptor for isoproterenol before and after desensitization was examined by measuring [³H]DHA displacement by isoproterenol. The EC₅₀ for isoproterenol in control tissue was 4.1 × 10⁻⁶ M, which did not differ importantly from 3.4 × 10⁻⁶ M for tissue exposed to isoproterenol for 30 minutes (mean of three experiments).

Adenylate Cyclase Activity

In many systems, activation of receptor-coupled adenylate cyclase is an essential step in the physiological expression of hormone interaction with tissue. To examine the possibility that alterations in adenylate cyclase activity would correlate with the inotropic desensitization that was observed, the adenylate cyclase response to isoproterenol was measured before and after 30 minutes exposure to 1 μM isoproterenol (Table 1). In control tissue, isoproterenol increased adenylate cyclase activity...
TABLE 1  Adenylate Cyclase Activity of Embryonic Chick Ventricular Homogenates

<table>
<thead>
<tr>
<th>Stimulating agent</th>
<th>Control (pmol cAMP/mg per 10 min)</th>
<th>After incubation (pmol cAMP/mg per 10 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>Increase from basal activity</td>
</tr>
<tr>
<td>A Basal</td>
<td>402 ± 51</td>
<td>606 ± 93</td>
</tr>
<tr>
<td>B Isoproterenol</td>
<td>1008 ± 122</td>
<td>2307 ± 273</td>
</tr>
<tr>
<td>C Propranolol</td>
<td>421 ± 52</td>
<td>19 ± 15</td>
</tr>
<tr>
<td>D NaF</td>
<td>2709 ± 316†</td>
<td>1930 ± 51‡</td>
</tr>
<tr>
<td>E Gpp(NH)p</td>
<td>1900 ± 51†</td>
<td>1528 ± 376</td>
</tr>
<tr>
<td>F Isoproterenol + Gpp(NH)p</td>
<td>3887 ± 362†</td>
<td>3485 ± 331†</td>
</tr>
<tr>
<td>F - E (Isoproterenol + Gpp(NH)p - (Gpp(NH)p)*</td>
<td>1957 ± 375</td>
<td></td>
</tr>
</tbody>
</table>

Both the absolute amount and increase above basal activity of adenylate cyclase before and after 30 minutes of incubation with 1 μM isoproterenol are shown. Values are mean ± SEM of six experiments.

* Increase from basal activity for Gpp(NH)p stimulation was subtracted from that for Gpp(NH)p + isoproterenol stimulation; ‡ Indicates differences from basal activity that are significant (P < 0.01); † Indicates a significant difference (P < 0.01) in activity before and after incubation with the agents indicated.

from the basal level of 402 ± 51 to 1008 ± 122 pmol/mg per 10 min. However, after 30 minutes of incubation with 1 μM isoproterenol and thorough washing, the response to subsequent isoproterenol exposure was significantly diminished to 627 ± 56 pmol/mg per 10 min (P < 0.01). The augmentation over basal activity was only 29% as great after 1 μM isoproterenol incubation as without incubation. Addition of propranolol caused no significant change in basal activity, indicating that there was no residual isoproterenol after washing. The response to NaF, which activates adenylate cyclase independently of the β-adrenergic receptor, was also unaltered by isoproterenol incubation (Table 1).

To determine if the responsiveness to isoproterenol after 30 minutes of exposure to isoproterenol could be restored by Gpp(NH)p, the increase in adenylate cyclase activity in response to Gpp(NH)p was subtracted from the increase in activity observed with isoproterenol + Gpp(NH)p. This difference reflects the adenylate cyclase response to isoproterenol when the guanyl nucleotide regulatory site is occupied by Gpp(NH)p. As indicated in Table 1, exposure 1 μM isoproterenol did not alter this augmentation in activity. Thus, Gpp(NH)p does restore responsiveness to isoproterenol after agonist-induced desensitization has occurred.

Isoproterenol concentration-adenylate cyclase activity curves, before and after 30 minutes of exposure to 1 μM isoproterenol, are shown in Figure 4. At every concentration of isoproterenol greater than 0.1 μM, the adenylate cyclase activity prior to incubation with 1 μM isoproterenol was greater than that after exposure. However, the concentration of isoproterenol that elicited a half-maximal response of adenylate cyclase differed relatively little (3 x 10^-7 vs. 8 x 10^-7 M) before and after exposure to isoproterenol for 30 minutes. It is of interest that although EC50 differ by an order of magnitude for contractility and adenylate cyclase activation, the relative increases in EC50 after exposure to isoproterenol are quite similar. These increases in EC50 are modest in magnitude, and the biological importance of these small changes is not certain.

Discussion

The phenomenon of hormonal regulation of receptors and target cell response is of fundamental biological importance and is an area of active current interest (Catt et al., 1979). In 1962, Murad and coworkers (1962) established that β-adrenergic agonists stimulate cAMP production in the canine heart. In other animal models, it has been found that CAMP responsiveness is diminished in the
failing heart (Sobel et al., 1969). In humans with chronic heart failure, urinary excretion of catecholamines is increased, and recently Thomas and Marks (1978) observed that, in patients with congestive heart failure, plasma catecholamine concentrations are elevated and peripheral blood lymphocytes have an attenuated cAMP response to isoproterenol. Taken together, these findings suggest that reduced cardiac responsiveness to endogenous catecholamines may be a pathophysiological mechanism in heart failure and supports the long-held clinical suspicion that tachyphylaxis to endogenous catecholamines may be a pathophysiological mechanism in heart failure and supports the long-held clinical suspicion that tachyphylaxis to endogenous catecholamines.

The mechanism of this suspected desensitization of the heart to catecholamines is not well understood, and this has prompted the present study of altered psychological and biochemical events occurring as a result of short-term in vitro exposure of embryonic chick ventricular tissue to the β-adrenergic agonist isoproterenol.

The observations reported here indicate that short-term (30-minute) exposure of chick embryo ventricular tissue does indeed result in a substantial reduction in inotropic responsiveness to subsequent challenge with isoproterenol. This is associated with a substantial reduction in maximal adenylyl cyclase response to isoproterenol but without alteration in the density of β-adrenergic receptors and only a modest reduction in receptor affinity. The decrease in the contractile response to β-adrenergic agonist exposure is not due to alteration in the contractile machinery or to deterioration of the preparation, because the basal contractility and the responsiveness to the positive inotropic effect of increased superfusate Ca²⁺ concentration is not diminished. Furthermore, the desensitization occurs after incubation with isoproterenol but not after a positive inotropic intervention (increased perfusate [Ca²⁺]) that acts through a nonadrenergic mechanism. Thus, there appears to be specific desensitization to the contractile and adenylyl cyclase activation effects of a β-adrenergic agonist in this system.

A desensitizing incubation with isoproterenol produces little change in the concentration of isoproterenol required to produce a half-maximal contractile response, although the absolute magnitude of the contractile effect is reduced substantially. This indicates that a 30-minute incubation with isoproterenol produced at most a modest decrease in the affinity of the β-adrenergic receptor for isoproterenol. Determination of the Kᵦ by antagonist binding studies is consistent with this, although antagonist binding properties need not, of necessity, closely reflect those of an agonist (Williams and Lefkowitz, 1977). However, the lack of change in agonist affinity as determined by antagonist displacement also is consistent with the physiological observation that the EC₅₀ for the contractile response changes little after a desensitizing incubation with isoproterenol.

Our finding of diminished adenylyl cyclase responsiveness to receptor stimulation after incubation of the tissue with isoproterenol to 29% of control response is concordant with the observed desensitization to the contractile effects of isoproterenol to 35% of control responses. Although the contractility response of the intact tissue was an order of magnitude more sensitive to isoproterenol than the adenylyl cyclase activity response of the tissue homogenate, the isoproterenol concentration-adenylyl cyclase response curve was qualitatively similar to that for contractility, with modest increases in the isoproterenol concentration that elicited half-maximal stimulation of both responses after incubation with isoproterenol. Incubation with isoproterenol did not alter basal adenylyl cyclase activity, and assay in the presence of propranolol confirmed that no significant isoproterenol effect remained following the wash after incubation. The adenylyl cyclase response to a non-β-adrenergic stimulus, NaF, was unaltered, indicating that the catalytic site of adenylyl cyclase was intact.

We found that the guanyl nucleotide analogue Gpp(NH)p activated adenylyl cyclase to the same degree before or after 30 minutes exposure to isoproterenol, as Dibner and Molinoff (1979) recently observed in rat cerebral cortex. This indicates that desensitization does not irreversibly alter the guanyl nucleotide regulatory site or its coupling to adenylyl cyclase. Furthermore, we found that, when the tissue was stimulated with isoproterenol in the presence of Gpp(NH)p, the desensitization to isoproterenol was abolished. This reversal of apparent agonist-induced uncoupling of the receptor from adenylyl cyclase is consistent with the observations of Harden et. al. (1979) and Mukherjee and Lefkowitz (1976) for frog erythrocytes and concordant with their model in which occupancy of the nucleotide regulatory site leads to conformational alterations that reactivate the desensitized receptor-enzyme complex.

Adenylyl cyclase-coupled β-adrenergic receptors have been identified directly using ligand binding techniques in numerous tissues, including cardiac tissue from the rat (Harden et al., 1976) and dog (Alexander et al., 1975) and in cultured myocardial cells from chick embryos (Tsai and Chen, 1978). Desensitization of cardiac tissue in vitro to the inotropic effects of catecholamines has not been described previously, although there are a number of hormone-receptor systems in which biochemical evidence of desensitization after both in vivo and in vitro exposure to catecholamines has been reported (Wolfe et al., 1977; Terasaki et al., 1978). Biochemical evidence for desensitization to catecholamines has been attributed to alterations at various sites in the β-adrenergic receptor-adenylyl cyclase system. In most tissues that have been investigated, tachyphylaxis to the effect of an adrenergic agonist has been accompanied by an unchanged affinity for agonist but with a decrease in apparent number of
β-adrenergic receptors as assessed by antagonist binding (Keabian et al., 1975; Mukherjee et al., 1975; Mickey et al., 1975). However, in the C6-2B glioma cell line, Terasaki and coworkers (1978) found catecholamine-induced desensitization of adenylate cyclase with no change in apparent receptor affinity or number. In the 1321NI astrocytoma cell line, no change in receptor number and a rapid uncoupling of the β-adrenergic receptor from adenylate cyclase was found (Harden et al., 1979). Recently, Tse et al. (1979) found that, in rats given isoproterenol chronically to include cardiac hypertrophy, there was a decrease in numbers of β-adrenergic receptors in the hypertrophied hearts, associated with diminution in contractile response to isoproterenol and decreased basal and stimulated adenylate cyclase activity. This desensitization to chronic catecholamine administration might be attributed partly to structural changes that may accompany isoproterenol-induced hypertrophy (Stan ton et al., 1969; Rona et al., 1959). Many other studies that report decreases in numbers of receptors with exposure to β-adrenergic agonists used substantially longer periods of exposure to agonist than the 30 minutes of incubation used in the present investigation. With more prolonged exposure, other mechanisms of desensitization may come into play, including endocytosis of the receptor complex as suggested by Chuang and Costa (1979) and as documented in other effector-receptor interactions (Catt et al., 1979). Thus, the short-term desensitization to the inotropic effect of isoproterenol reported here, accompanied by uncoupling of the receptor from adenylate cyclase activation, may be the initial step in a sequence of events resulting in long-term desensitization and actual loss of receptors from the cell surface.

In conclusion, we have shown that, in chick embryonic myocardium, desensitization to the contractile effect of β-adrenergic stimulation can be induced by exposure to a β-adrenergic agonist. There is a concomitant decline in the response of adenylate cyclase to β-adrenergic agonist stimulation. The decrease in adenylate cyclase response can be abolished completely by occupation of the guanyl nucleotide regulatory site. There is no associated alteration in β-adrenergic receptor number and little change in affinity. These observations are consistent with agonist-induced uncoupling of the interaction between β-receptor and adenylate cyclase and may reflect an important mechanism for the short-term modulation of the response of cardiac tissue to catecholamines.

References


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Ionic Currents during Hypoxia in Voltage-Clamped Cat Ventricular Muscle

ARThUR VLEUGELS, JOHAN VERECKE, AND EDWARD CARMELIET

SUMMARY To explore the mechanisms underlying the shortening of the cardiac action potential in hypoxia, we studied the effect of hypoxia on the ionic currents in cat papillary and trabecular muscles using the single sucrose gap-voltage clamp technique. For potentials positive to -70 mV, hypoxia induces an increase in time-independent outward current. In the slow inward current, the amplitude of the slow inward current is not affected by hypoxia. Its time constant of inactivation appears slightly decreased. The prolongation of the action potential by epinephrine during hypoxia is accompanied by an increase in the slow inward current. As a result of these studies, we conclude that the shortening of the cardiac action potential in the early stage of hypoxia results from an increase in K+ outward background current. Circ Res 47: 501-508, 1980

IT HAS long been known that the most striking effect of hypoxia on the cardiac transmembrane potential is a shortening of the action potential. The effect was described first for cat papillary muscle (Trautwein et al., 1954) and has been demonstrated in a variety of cardiac muscle preparations. However, the actual mechanisms underlying this shortening still remain uncertain. Nevertheless, a knowledge of these mechanisms may be of interest since it may contribute to a greater understanding of the genesis of arrhythmias in the ischemic heart. Taking into account the characteristics of the different membrane currents that may contribute to the repolarization in heart cells (see, e.g., Carmeliet and Verecke, 1979), three particular currents are important in determining the action potential duration: (1) the slow inward current; (2) the time-dependent outward current; and (3) the background current. Changes in action potential duration may result from changes in the driving forces, the fully activated conductances, or the kinetics of these currents. In the earliest work on hypoxia, the shortening was attributed to an increase in K+ outward current (Trautwein et al., 1954; Trautwein and Dudel, 1956; Webb and Hollander, 1956; MacLeod and Daniel, 1965; Prasad and MacLeod, 1969). After voltage-clamp experiments revealed the existence and the role of the slow inward current (Rougier et al., 1969), the effect of hypoxia generally was accepted to result from a suppression of the latter current (e.g., Hunter et al., 1972; McDonald...
Evidence for uncoupling of the beta receptor-adenylate cyclase complex.

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