Metabolic and Circulatory Responses to Selective Adrenergic Stimulation and Blockade

By Donald C. Harrison, M.D., and John Robert Griffin, M.D.

Indirect Evidence has long supported the idea that catecholamines play an important role in fat metabolism. Direct evidence for this was obtained in 1956, when investigators, Dole and Gordon and Cherkas, working independently, demonstrated that the intravenous infusion of aqueous epinephrine in humans results in a significant rise in the level of plasma free fatty acids (FFA). This response has also been observed after the intravenous administration of norepinephrine and isoproterenol and has been successfully blocked by a variety of agents.

In 1948, Ahlquist proposed that two distinct adrenergic receptors, alpha and beta, mediate the neuromuscular responses to sympathomimetic amines. Subsequent work has confirmed this hypothesis, permitting classification of adrenergic agents on the basis of their neuromuscular activity. In contrast, investigations attempting to demonstrate that the metabolic responses to sympathomimetic amines are also mediated by alpha and beta receptors of the type proposed by Ahlquist have been confusing.

In 1962, Pilkington and associates demonstrated that administration of a new selective beta-adrenergic blocking agent, pronethalol, effectively blocked subsequent mobilization of FFA by epinephrine and that a well-known alpha-adrenergic blocking agent, phenoxybenzamine, failed to block this effect. With this evidence, Pilkington and co-workers suggested that the release of FFA by catecholamines might be mediated by beta-adrenergic receptors. Pronethalol has since been shown to be carcinogenic in mice and has been abandoned in favor of a safer and more potent beta-adrenergic blocking agent, propranolol.

In the present study, various agents with specific alpha- and beta-stimulating and blocking effects have been employed in an attempt to define more clearly the nature of the receptors which mediate the release of FFA.

Methods

Each patient in this study was hospitalized in the clinical research center and evaluated by means of a history, physical examination, cardiac series, and 12-lead electrocardiogram. The age, clinical diagnosis, and state of digitalization of each patient is shown in table 1.

Continuous, 15 to 19-minute, constant-rate infusions of the specific beta-adrenergic stimulating agent, isoproterenol, and the equally specific alpha-adrenergic agent, methoxamine, were administered respectively to eight and three subjects. The rate of infusion for each patient was based on body weight and individual dose-response sensitivity and ranged from 1.2 to 3.7 μg/min for isoproterenol and 0.5 to 1.0 mg/min for methoxamine. Six of the eight patients received two identical infusions of isoproterenol, one before and one after the administration of the specific beta-adrenergic blocking agent, propranolol, 100 μg/kg. Two subjects were given isoproterenol infusions both before and after the administration of the alpha-adrenergic blocking agent, phenoxybenzamine, 0.5 to 0.75 mg/kg/24 hr prior to the study.

Heart rate was determined in every patient from 30-second electrocardiographic tracings obtained at various intervals before, during, and after the infusion of each drug. Blood pressure was monitored by means of an external brachial arterial pressure cuff.

*Supplied by Dr. Alex Sahagian-Edwards, Ayerst Laboratories, New York, New York.
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Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, yr</th>
<th>Diagnosis</th>
<th>Digitalization*</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.V.</td>
<td>61</td>
<td>Rheumatic heart disease, with mild myocardial failure</td>
<td>+</td>
</tr>
<tr>
<td>K.T.</td>
<td>51</td>
<td>Rheumatic heart disease, with mitral stenosis, aortic insufficiency, and aortic stenosis, all mild</td>
<td>+</td>
</tr>
<tr>
<td>H.J.</td>
<td>54</td>
<td>Rheumatic heart disease, with mitral stenosis</td>
<td>+</td>
</tr>
<tr>
<td>S.A.</td>
<td>48</td>
<td>Cardiomyopathy</td>
<td>+</td>
</tr>
<tr>
<td>T.M.</td>
<td>37</td>
<td>Rheumatic heart disease, with mitral insufficiency</td>
<td>+</td>
</tr>
<tr>
<td>T.N.</td>
<td>52</td>
<td>Cardiomyopathy</td>
<td>+</td>
</tr>
<tr>
<td>M.G.</td>
<td>35</td>
<td>Rheumatic heart disease, with mitral stenosis and mitral insufficiency</td>
<td>+</td>
</tr>
<tr>
<td>F.S.</td>
<td>63</td>
<td>Rheumatic heart disease, with mitral insufficiency</td>
<td>+</td>
</tr>
</tbody>
</table>

*Patient receiving maintenance doses of digitalis glycosides.

Samples of venous blood for determination of plasma glucose and plasma FFA concentrations were withdrawn at designated intervals from an intravenous catheter, inserted into an antebrachial or antecubital vein 30 minutes prior to the study and kept patent by slow saline drip. Blood samples obtained in this manner were immediately mixed with a fluoride-oxalate powder and placed in crushed ice. Within 2 hours, the plasma was separated by refrigerated centrifugation at 4°C for 10 minutes at 2,500 rpm and frozen immediately. Subsequent determinations of plasma glucose and plasma FFA were performed 2 weeks to 4 months later.

Two methods were used to determine plasma FFA. A slight modification of the microtitration technique first described by Dole was used to determine plasma FFA concentrations in samples from the first six patients. For the remaining determinations, the newer, more rapid, colorimetric method described by Duncombe was used. In this method, a small aliquot of plasma is shaken thoroughly with a mixture of isopropyl ether and silicic acid powder. Phospholipids, which interfere with the colorimetric determination, are absorbed by silicic acid, while FFA and other noninterfering plasma lipids are extracted by the ether without being absorbed. A known aliquot of ether is then evaporated to dryness and the residue reconstituted to its original volume with chloroform. An aqueous copper reagent, formed by complexing copper nitrate with triethanolamine and acetic acid, is layered over the chloroform, and the two phases are shaken vigorously into an emulsion; the FFA in the chloroform forms a complex with the copper reagent in stoichiometric relationship. The two phases are allowed to separate, and the excess aqueous copper reagent is removed by suction. An aliquot of the chloroform is then pipetted into a spectrophotometric tube and a characteristic, yellow-brown color is developed with diethylthiocarbamate reagent. Optical density is read against a reagent blank at 440 millimicrons to three significant figures. FFA concentration may then be determined by use of the regression line derived by Duncombe. In the present study, when control samples were analyzed by both methods, agreement within 40 μEq/L was obtained.

Results

Isoproterenol Infusions

Results Following Control Infusions

In all eight isoproterenol infusions, the infusion rate produced a marked cardio-acceleration, used in this study as the criterion of adequate beta-adrenergic stimulation. The mean increase in heart rate was 82 ± 18 beats /min (fig. 1). The changes in blood pressure produced by beta-adrenergic stimulation before and after blockade with propranolol showed a slight but inconsistent decrease. In
Cardiac effects of beta-adrenergic stimulation with isoproterenol before and after the administration of propranolol. The mean and standard deviation of the heart rates during a control period (CONT.), during isoproterenol infusion (INF.), and 45 minutes after infusion (45') are shown. In the left panel, the results prior to the administration of propranolol (BEFORE PROP) are shown. In the right panel, the values obtained following administration of propranolol (AFTER PROP) may be seen.

Results Following Alpha-Adrenergic Blockade with Phenoxybenzamine

Two patients were given 100 and 45 mg of phenoxybenzamine, respectively, until obvious signs of orthostatic hypotension intervened (170/95 to 110/50 and 130/75 to 105/60, respectively), this being the criterion of effective alpha-adrenergic blockade in this study. In both cases, subsequent isoproterenol infusion produced significant cardio-acceleration, with increases of 56 and 85 beats/min, and a marked increase in plasma FFA level, with rises of 460 and 1,220 μEq/L (fig. 4). Blood glucose levels were unaltered by the infusions (fig. 4).

Results Following Beta-Adrenergic Blockade with Propranolol

Both cardio-accelerator and FFA responses to isoproterenol were virtually abolished by the prior administration of propranolol. The mean increase in heart rate was reduced to 13 ± 11 beats/min (fig. 1) and the rise in plasma FFA dropped sharply to 160 ± 30 μEq/L (fig. 5). Again, blood glucose was unchanged by infusion (fig. 3).

Methoxamine Infusions

Results Following Control Infusions

In all three patients who received methoxamine infusions, sharp rises in systemic arterial pressure were observed, with a mean rise in systolic pressure of 62 ± 32 mm Hg. A small decrease in heart rate was also noted in each patient, with a mean decrease of 13 ± 3 beats/min (fig. 6). Both FFA and glucose levels were unaffected by the infusions (figs. 6 and 7).

Discussion

The results of this study strongly support the hypothesis that adrenergic control of...
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Blood glucose response to isoproterenol infusion before and after propranolol. The mean and standard deviations of the blood glucose concentrations before administration of propranolol are represented by the solid circles and after administration of propranolol by the solid squares. ISO-INF represents the period of infusion of isoproterenol.

Failure of alpha-adrenergic blockade with phenoxybenzamine to affect the cardiovascular and metabolic responses to isoproterenol infusion. The responses of two patients to isoproterenol infusion following administration of phenoxybenzamine are shown. In panel A the response of plasma FFA to isoproterenol infusion (ISO) is shown. Control values are represented by (C) and values following infusion by [p (Inf.)]. TN and MG identify specific patients. In panel B, the response of heart rate to isoproterenol infusion is shown. In panel C, the response of blood glucose to isoproterenol is shown.

Plasma FFA concentration is mediated by means of a receptor mechanism in classic pharmacological terms. Isoproterenol, known on the basis of its neuromuscular activity to be a highly specific beta-adrenergic stimulation agent, produced a marked increase in plasma FFA concentration concomitant with its cardio-accelerator action. Both the cardio-acceleration and increase in plasma FFA level produced by isoproterenol were profoundly inhibited by the prior administration of propranolol, a specific beta-adrenergic blocking agent. In contrast, methoxamine, which has been shown on the basis of its neuromuscular action to be a highly specific alpha-adrenergic stimulating agent, produced no increases in plasma FFA concentration at infusion rates sufficient to cause pronounced vasopressor activity. In addition, prior blockade of the alpha-adrenergic system with phenoxybenzamine had no effect in altering either the increase in plasma FFA or the cardio-acceleration resulting from isoproterenol infusion. All of these findings are consistent with the hypothesis that adrenergic control of FFA release is mediated by beta-adrenergic receptors in the system of classification proposed by Ahlquist.

Aromatic amines not generally considered to exhibit beta-adrenergic stimulating properties may also increase circulating FFA by

**Figure 3**

**Figure 4**

**Figure 5**

*Circulation, Volume XXXIV, August 1966*
the clearly demonstrated property of many of these amines to effect the release of endogenous stores of norepinephrine.\(^{18-21}\) Norepinephrine released in this manner may then be active in producing a rise in plasma FFA and lead to a confusing picture.

The use of highly specific, alpha- and beta-adrenergic stimulating and blocking agents in this study would appear to permit limited speculation regarding their structure-activity relationships. The structure of the four compounds used in this study are shown in figure 8. Norepinephrine (fig. 8C) is known to have both alpha- and beta-adrenergic stimulating effects. The addition of a large chemical radical to the amine end of this compound appears to destroy its alpha-adrenergic stimulating properties and convert it into a pure beta-adrenergic stimulating agent, as represented here by isoproterenol (fig. 8A). In contrast, removal of the hydroxy-groups from the phenyl end of norepinephrine or substitution of these groups with non-ionizing radicals appears to destroy the beta-adrenergic stimulating activity of this compound and convert it into a pure alpha-adrenergic stimulating agent, here represented by methoxamine (fig. 8B).

Adrenergic blocking compounds appear to be formed by the addition of large, interfering radicals to the structurally “active” areas of the phenylethylamine skeleton or a similar congener. Thus, the beta-adrenergic blocking agent, propranolol, is characterized by the addition of a second aromatic ring to the phenyl end of the compound, blocking the “active” hydroxy-positions (fig. 8D). The methoxy radicals on the phenyl end of methoxamine may be responsible for the recently demonstrated, beta-adrenergic blocking property of this compound.\(^{22}\) Substitution of large, interfering radicals to the amine end

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**Figure 6**

Cardiovascular and blood glucose responses to methoxamine infusion. In the top panel, the changes in systolic arterial pressure (SYST. ART. PRES.) produced by methoxamine infusion (METH) are shown for three patients. In the center panel, the changes in heart rate (H.R.) are depicted. In the bottom panel, the blood glucose response is shown.

**Figure 7**

FFA response to methoxamine infusion. The plasma FFA concentrations before and after methoxamine infusion (METH. INF.) are shown for three patients.
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Figure 8
Structure of pharmacological agents used in these studies. The active groups in each formula are represented by the heavily printed areas. For example, the amine radical of norepinephrine is considered the alpha-stimulating group.

of these compounds convert them into potent, alpha-adrenergic blocking agents.23, 24 In the case of the halo-alkylamines, a highly reactive intermediate at the amine end of the molecule is thought to be responsible for blockade of the alpha receptors.25, 26 An example of such a compound is shown by the structure of phenoxybenzamine (fig. 8E).

It is tentatively concluded from such speculation that the alpha-adrenergic stimulating effects of adrenergic amines appear to depend on the functional integrity of the amine radical of these compounds, whereas the beta-adrenergic stimulating effects of sympathomimetic agents seem to depend on the presence of hydroxyl or similar functional groups on the phenyl ring in a 3 and a 4 position. The suggestion has been made that adrenergic receptors be renamed according to the functional groups which they receive.24 Thus, using norepinephrine as a prototype, the alpha-adrenergic receptors might be renamed "amine-receptors," and the beta-adrenergic receptors might be called "hydroxy-receptors." The findings of this study support this concept and extend it to include the adrenergic control of FFA release.

Although other investigators have noted variable changes in blood glucose following isoproterenol infusion, no changes were observed in our patients. Thus, no clear relationship between an adrenergic mechanism and glucose release can be concluded from this study.

Summary
Selected cardiovascular and metabolic responses to highly specific alpha- and beta-
adrenergic stimulation and blockade were studied in eight patients. Selective beta-adrenergic stimulation with isoproterenol produced a rise in the level of plasma FFA concomitant with its cardio-accelerator effect. Both of these responses were inhibited by the prior administration of the specific beta-adrenergic blocking agent, propranolol. Selective alpha-adrenergic stimulation with methoxamine increased systemic arterial pressure but produced no metabolic changes. Alpha-adrenergic blockade with phenoxybenzamine did not alter the circulatory or metabolic responses to isoproterenol. These findings support the hypothesis that the adrenergic influence over the level of circulating FFA is mediated by the beta-adrenergic receptors. Structure-activity relationships of the four agents used in this study have been examined.

Acknowledgment

Our appreciation to Sue Pirages and Sandra Ridges for their help in this study is acknowledged. The Nursing Staff of the Clinical Research Center kindly helped with many aspects of this study. The suggestions of Dr. John W. Farquhar were helpful in initiating these studies.

References

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Circulation. 1966;34:218-225
doi: 10.1161/01.CIR.34.2.218

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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