Effect of the Endeca-peptide Eledoisin on the Coronary Blood Flow: Comparison with Nitroglycerin, Bradykinin and Epinephrine in the Dog

By Mario Bergamaschi, V.M.D., and Alfredo H. Glässer, M.D.

In 1961 Erspamer and Anastasi\(^1\) isolated from the posterior salivary glands of Eledone (moschata and Aldrovandi) a new polypeptide, which was identified as the endeca-peptide Pyr-Pro-Ser-Lys-Asp(OH)-Ala-Phel-Gly-Leu-Met-NH\(^2\).

The subsequent synthesis of Eledoisin confirmed the above constitution and aminoacid sequence.\(^2\) In their short report, Erspamer and Anastasi described some pharmacological properties of Eledoisin. Eledoisin displays a potent vasodilator action on several vascular beds; it decreases blood pressure in some species of animals and increases it in others.\(^3\) In general, Eledoisin stimulates extravascular smooth muscle.\(^4\)

The present study was undertaken to investigate the actions of Eledoisin on the coronary blood flow, heart rate, and myocardial contractility. Nitroglycerin, bradykinin, and \(\beta\)-epinephrine, whose cardiovascular actions are well known, were used for comparison.

**Methods**

Mongrel dogs of both sexes, weighing from 10 kg to 15 kg were used. Anesthesia was induced with 25 mg/kg of thiopentone sodium injected iv followed by 80 mg/kg of chloralose iv.

**ARTERIAL BLOOD PRESSURE**

Systemic blood pressure was recorded by an Elema electromanometer connected to the femoral artery by a polyethylene catheter. It was possible therefore to record mean, systolic, and diastolic pressure. The mean blood pressure was calculated as arithmetic mean pressure.

**CORONARY BLOOD FLOW**

The trachea was cannulated and the lungs were inflated by a Starling Ideal Pump. Under artificial respiration the chest was opened on the left side by removing the fourth and fifth ribs. The pericardium was then incised and the edges were sewed to the chest wall to bring the heart to an easily accessible position. The descending branch of the left coronary artery, near to its origin from the main trunk, was carefully dissected and freed of all adherent extraneous tissues. Both the thermoelectronuhr of Rein\(^5\) and the recording rotameter of Shipley-Wilson\(^6\) were used to record coronary blood flow. The first method was used when drugs were given intravenously into a femoral vein; the second one when drugs were given intra-arterially into the anterior descending branch of the left coronary artery. When the thermostromuhr of Rein was used, a calibrated unit was applied around the isolated vessel. When the second method was used, the coronary bed was perfused with blood derived from the carotid artery, and the recording rotameter was inserted in the circuit. Two hundred IU/kg of heparin were given at the beginning of the experiment and, during the experimental period, heparin was given at the rate of 100 IU/kg every hour. Intracoronary injections of drugs were made into an air bubble trap placed in a small lateral branch of the tube leading from the meter to the heart. Each dose was dissolved in 0.5 ml of saline or water and injected in 10 seconds; control injections of an equal volume of both solutions resulted in no significant alterations in coronary flow. No change of flow was considered significant unless it was greater than 10%.

**MYOCARDIAL CONTRACTILITY AND HEART RATE**

Recordings of the changes in myocardial contractility and heart rate were obtained by means of a modified strain-gauge arch\(^7\) sutured directly to the myocardium of the right or left ventricle in a line from the base to the apex, near the interventricular septum. The amplitude of our strain-gauge records was proportional to the force of contraction as in the experiments performed with the Walton strain-gauge.\(^8\),\(^9\) Arterial blood pressure, coronary blood flow, and heart contractile force were recorded simultaneously by three mirror galvanometers and a photokymograph.
DRUGS

The following preparations were used: bradykinin (Sandoz); \( L \)-epinephrine (R.P.); nitroglycerin at 5% in ethanol (C.Erba); Eledoisin as a highly purified extract (Erspamer-Anastasi) or as a synthetic product (Sandoz); papaverine chloride (C.Erba); heparin (Liquemin: Roche); thiopentone sodium (Farmotal: Farmitalia), and chloralose (Merck).

Results

1. BLOOD PRESSURE

Eledoisin

Rapid intravenous injections of Eledoisin cause short-lasting hypotension. Doses of 0.0004 and 0.002 \( \mu g/kg \) decreased the mean arterial blood pressure by 11 and 23 mm Hg, while higher doses, 0.02, 0.04, and 0.4 \( \mu g/kg \) reduced the blood pressure by 41, 46, and 47 mm Hg. Figure 1 shows that even at a dose 1000 times the minimal active dose, Eledoisin is not able to lower blood pressure under a minimum of 70 mm Hg. Systolic and diastolic pressures are reduced by Eledoisin. As the diastolic drop is higher than the systolic, the pulse pressure increases. This effect is quite clear at 0.02 and 0.04 \( \mu g/kg \). A reduction in pulse pressure is observed only at the highest dose, 0.4 \( \mu g/kg \).

Arterial blood pressure starts to decrease 11 to 15 seconds after the injection, reaches its lowest point a few seconds later, and returns to the control level within a few minutes.

Nitroglycerin

Nitroglycerin behaves like Eledoisin (fig. 2). Blood pressure changes are similar. The minimal active dose on the arterial blood pressure is 1 \( \mu g/kg \). A dose 100 times higher reduces mean blood pressure to 56 mm Hg.

\( L \)-Epinephrine (fig. 3)

At the smallest doses, 0.01, 0.05, and 0.1 \( \mu g/kg \), \( L \)-epinephrine slightly decreases mean blood pressure. At higher dosages, 0.2 and 0.5 \( \mu g/kg \), it decreases diastolic pressure more than the systolic, causing an increase in pulse pressure.
EFFECTS OF ELEDOSIN ON CORONARY FLOW

2. HEART RATE AND MYOCARDIAL CONTRACTILITY

Eledoisin

A slight and transient positive chronotropic and inotropic effect, 12% and 13%, is observed following the intravenous injection of Eledoisin (fig. 1). This effect becomes manifest only when high doses of Eledoisin are used, 0.02, 0.04, and 0.4 μg/kg. It may well be caused by a reflex mechanism as it follows the decrease of the systemic blood pressure and reaches a maximum value when blood pressure is at its lowest point.
Nitroglycerin

Nitroglycerin (fig. 2) acts like Eledoisin at low doses, 1 and 10 μg/kg, but at higher doses, 100 μg/kg, provokes a slight and transient reduction of the heart rate and of the myocardial contractility beneath the control values.

Bradykinin

Bradykinin provokes a moderate increase in heart rate (20%) and in myocardial contractile force (15%) only at the dose of 0.5 to 1 μg/kg. Heart rate and myocardial contractility return to the control values more rapidly than after Eledoisin.

\( l - \)Epinephrine

\( l - \)Epinephrine increases myocardial contractility with the usual dose-response relationship, (fig. 3), from a minimum of 4% at 0.01 μg/kg, to a maximum of 58% at 0.5 μg/kg. There are no significant changes in heart rate at the low doses of \( l - \)epinephrine; a slight tachycardia appears only at higher doses.

3. CORONARY BLOOD FLOW. (A) Intravenous Injection

Eledoisin

Eledoisin (fig. 4 and table 1) increases coronary blood flow even when blood pressure decreases. The rise in coronary flow begins a few seconds before any change in blood pressure, heart rate or myocardial contractility; generally it reaches its maximum within 20 seconds and returns to the initial level in a few minutes. In some experiments we observed fluctuations in the coronary flow, particularly when systemic blood pressure was at its lowest point.

The minimal active dose on the coronary flow, 0.0004 μg/kg (table 1) is also the minimal active dose on blood pressure. The rise of the flow is greater and lasts longer at higher doses, but in any case it did not last longer than ten minutes. Some experiments, performed with continuous intravenous infusion of Eledoisin, show that there is a slight decrease of the blood pressure, with a more consistent decrease of diastolic pressure, even at the minimal active dose on the blood flow, 0.001 and 0.005 μg/kg per minute.

Nitroglycerin

Results obtained with nitroglycerin (table 1) clearly indicate a similarity with Eledoisin. The minimal active dose is 1 μg/kg; at higher doses the increase in blood flow is more evident but in some experiments, a secondary transient drop, under the control level, occurs.

Bradykinin

Coronary blood flow increased following injection of doses of bradykinin as low as 0.05 μg/kg. The effect on coronary blood flow became manifest a few seconds earlier than changes in blood pressure. Smaller doses of Eledoisin produced similar but more long-lasting changes. The notch at the top of the
EFFECTS OF ELEDOISIN ON CORONARY FLOW

Effect of intravenous injection (femoral vein) of bradykinin (1 μg/kg). As blood pressure decreases coronary blood flow is depressed briefly while a moderate increase in heart rate and myocardial contractility occur.

A curve representing coronary blood flow following 1 μg/kg of intravenous bradykinin (fig. 5) is typical of the effects of both bradykinin and Eledoisin. Bradykinin seems to be about 100 times less active than Eledoisin.

**TABLE 1**

<table>
<thead>
<tr>
<th>Dose μg/kg</th>
<th>No. of exper.</th>
<th>Average peak effect</th>
<th>Average duration of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eledoisin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0004</td>
<td>8</td>
<td>5.5 ± 0.5</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>0.004</td>
<td>14</td>
<td>9 ± 1.24</td>
<td>49 ± 7</td>
</tr>
<tr>
<td>0.02</td>
<td>9</td>
<td>9 ± 0.94</td>
<td>87 ± 27</td>
</tr>
<tr>
<td>0.04</td>
<td>9</td>
<td>22 ± 4.24</td>
<td>307 ± 210</td>
</tr>
<tr>
<td>0.4</td>
<td>8</td>
<td>38 ± 2.48</td>
<td>361 ± 256</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>6 ± 1.73</td>
<td>49 ± 11</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>12.6 ± 1.74</td>
<td>64 ± 11</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>17 ± 0.95</td>
<td>75 ± 11</td>
</tr>
<tr>
<td>50</td>
<td>7</td>
<td>14.5 ± 1.28</td>
<td>84 ± 19</td>
</tr>
<tr>
<td>100</td>
<td>4</td>
<td>14.5 ± 1.32</td>
<td>138 ± 11</td>
</tr>
<tr>
<td>Bradykinin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>3</td>
<td>2.5 ± 0.25</td>
<td>10 ± 0.16</td>
</tr>
<tr>
<td>0.1</td>
<td>5</td>
<td>3.5 ± 0.91</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>9 ± 0.58</td>
<td>40 ± 14</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>12 ± 1.78</td>
<td>69 ± 10</td>
</tr>
<tr>
<td>L-Epinephrine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.01</td>
<td>5</td>
<td>0.6 ± 0.91</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>1.8 ± 2.75</td>
<td>41 ± 6</td>
</tr>
<tr>
<td>0.1</td>
<td>7</td>
<td>14.5 ± 1.78</td>
<td>63 ± 16</td>
</tr>
<tr>
<td>0.2</td>
<td>14</td>
<td>14 ± 0.64</td>
<td>68 ± 6</td>
</tr>
<tr>
<td>0.5</td>
<td>7</td>
<td>22 ± 3.25</td>
<td>76 ± 15</td>
</tr>
</tbody>
</table>

*The increase of coronary blood flow (average peak effect) is expressed as mm deflection of the galvanometer.

†The duration of effect (average duration of effect) is expressed in seconds.

± = standard deviation of the mean.

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Effects of intra-arterial injections of Eledoisin (0.00002 μg/kg) (Anterior descending branch of the left coronary artery). Coronary blood flow increases without any change in systemic blood pressure, myocardial contractility or heart rate.

Coronary Blood Flow: (B) Intracoronary Injection

Under the conditions of these experiments, the volume of blood passing through the anterior descending branch of the left coronary artery is 40 to 60 ml/min. There is a clear relationship between coronary blood flow and blood pressure; when blood pressure increases or decreases there is a corresponding rise or fall in coronary blood flow.

Eledoisin, nitroglycerin and papaverine injected into the coronary artery, increased coronary blood flow, but not always was there a satisfactory dose-response relationship, and the response varied from animal to animal. Eledoisin, in doses ranging from 0.00002 to 0.002 μg, produced short-lasting 20% to 60% increases of coronary flow without any modification of heart rate, myocardial contractility or blood pressure (fig. 6).

Higher doses of Eledoisin, 0.02 to 0.2 μg, increased coronary blood flow up to 80%. However, when Eledoisin reached the systemic circulation and produced hypotension, blood flow no longer increased and even decreased.

Nitroglycerin at the dose of 1 μg increased coronary blood flow 12%, at 2 μg, 23%, at 5 μg, 62%, and at 10 μg only 22%.

Papaverine, at the dose of 500 μg, increased coronary blood flow 67% while at the dose of 1 mg it produced an increase of the blood flow ranging from 62% to 105%.

Discussion

Our results show that the newly described natural endopeptide Eledoisin increases coronary blood flow without affecting essentially heart rate or myocardial contractility. In this respect Eledoisin behaves like nitroglycerin but differs from epinephrine, which increases coronary blood flow and heart contractility even at hypotensive dosages.

Both Eledoisin and nitroglycerin increase coronary blood flow independently of blood pressure changes, as is clearly shown by experiments in which drugs were injected directly into the coronary arteries. Eledoisin and nitroglycerin injected intravenously, increase, for a short time, coronary blood flow even when blood pressure drops markedly.

It is rather difficult to dissociate coronary blood flow increase from blood pressure decrease; even at the minimal active dose on the
coronary flow, both Eledoisin and nitroglycerin slightly decreased diastolic pressure. These results are in agreement with the fact that Eledoisin and nitroglycerin are potent vasodilators. Bradykinin increases coronary flow too. However, in comparison to Eledoisin, it seems to have a minor and shorter action on the coronary blood flow. Bradykinin appears to be 100 times less active than Eledoisin.

The similarity between Eledoisin and nitroglycerin is not observed at all dosages. Nitroglycerin, at a dosage 100 times the minimal active dose on blood flow, reduces heart rate and myocardial contractility slightly and for a short time, and blood flow increase may be followed by a transient decrease.

There is considerable evidence from experimental animals, that nitrites, including nitroglycerin, increase coronary blood flow by direct action on the coronary vascular bed.

Our findings appear to indicate that Eledoisin acts like nitroglycerin; of course, it would be necessary to investigate coronary arteriovenous $O_2$ difference and myocardial $O_2$ consumption in order to elucidate this point.

Our results do not explain how $L$-epinephrine increases coronary blood flow at doses that provoke a moderate fall in blood pressure without affecting heart rate. The rise of coronary flow may be related to an increased myocardial contractility. However, the importance of the extravascular factors remains to be determined.

Summary

The effects on coronary blood flow of the endocapeptide Eledoisin have been studied in the anesthetized dog. Eledoisin increases coronary blood flow without increasing myocardial contractility and heart rate. Nitroglycerin and the polypeptide bradykinin increase coronary blood flow in the same way, but are markedly less active than Eledoisin. $L$-Epinephrine increases coronary blood flow, but at the same time produces a stimulating action on the myocardium.

Acknowledgment

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References


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