Determination of Infused Epinephrine and Norepinephrine in Plasma Using Isolated Guinea Pig Heart Technique

By Walter M. Booker, Ph.D., Tracy Walton, Rafael Linares, and Andre F. De Schaepdryver, M.D.

The use of the isolated perfused guinea pig heart as a method of assaying catecholamines in aluminum oxide extracts of urine, tissues, and plasma has been reported previously. The isolated guinea pig heart preparation proved to be the most sensitive of the various biological assay methods available for this purpose.

It is a well-known fact that physicochemical methods are extremely limited in their reliability with regard to the estimation of the very low concentrations of catecholamines in plasma, except when labeled compounds are employed.

We have extended some of our earlier observations regarding the use of the isolated perfused guinea pig heart as an assay method for the estimation of plasma levels of catecholamines after intravenous infusion in dogs. In the first series of experiments, the method was re-evaluated with regard to sensitivity and specificity and adapted to a procedure of differential estimation of epinephrine and norepinephrine, by using dichloroisoproterenol (DCI) as a blocking agent. We have already reported in preliminary experiments that DCI blocks the epinephrine effect on the heart ahead of the norepinephrine effect where repeated or successive testing of the two substances is undertaken in the same experiment.

In the second series of experiments, dogs were infused with epinephrine and norepinephrine in separate experiments and blood samples were collected at various intervals before, during, and after the infusion. These samples were assayed for their catecholamine content on the isolated guinea pig heart, using plasma extracts as well as crude plasma.

Methods

The isolated perfused guinea pig heart technique has been described previously. While the method of testing aluminum oxide extracts of plasma, tissues, and urine has been described in detail in the reference cited, it may be of value to indicate here that in the first experiments, involving the application of this method of assay to experimental and clinical testing during the period of work in the laboratory of Professor C. Heymans, Ghent, Belgium, "blind" aluminum oxide samples from plasma, tissues, and urine were sent to the senior author of this paper by Dr. A. De Schaepdryver, who performed the extractions and ran spectrofluorometric analyses on aliquots of the same samples. The spectrofluorometric concentrations of the catecholamines of the samples were revealed only after the biological assay reports had been given. There was greater than 95 per cent agreement. In the procedures described here, catecholamines were added to heated plasma, to distilled water, and to heated urine, and extracts were made as described below. Our recoveries have averaged 70 per cent, which is the figure most widely experienced by workers in this field. In this laboratory, we have also compared the spectrofluorometric analysis of low concentrations of catecholamines with the biological assay method. We have found that, while we get definitive positive inotropic responses at concentrations as low as 0.0004 µg. per cc., this concentration is not distinctly and dependably measurable on the spectrofluorometer. In higher concentrations of the plasma analyzed, however, good agreement has been found between the spectrofluorometric and the biological assay findings.

The infusion experiments were performed on

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PLASMA CATECHOLAMINES

FIGURE 1
Responses of isolated perfused guinea pig heart to:
† 1: 0.0004 µg. of epinephrine
Inotropic response 21 per cent
† 2: 0.002 µg. of epinephrine
Inotropic response 46 per cent
† 3: 0.004 µg. of epinephrine
Inotropic response 64 per cent
† 1: 0.0004 µg. of norepinephrine
Inotropic response 15 per cent
† 2: 0.002 µg. of norepinephrine
Inotropic response 33 per cent
† 3: 0.004 µg. of norepinephrine
Inotropic response 56 per cent.
(See table 1.) Number of experiments in each group: 20.

adult mongrel dogs weighing between 10 and 20 Kg. and anesthetized with morphine (1 mg./Kg., subcutaneously) and chloralose (100 mg./Kg., intravenously). Arterial blood pressure was recorded from the right common carotid artery, using a Sanborn electromanometer.

Freshly prepared solutions of epinephrine and norepinephrine were infused for 30 minutes in a dose of 1.5 µg./Kg./min. into the left femoral vein by means of a constant-infusion apparatus, at a rate of 1 ml./min. Twenty-ml. blood samples were collected in cooled centrifuge tubes from the right femoral artery, before, during, and after the infusion. The samples were immediately centrifuged at low temperature, and the plasma removed for testing directly on the isolated guinea pig heart, and for extracting by means of adsorption on aluminum oxide, according to the method of von Euler and Floelling, modified by De Schueppdryver. Also, aluminum oxide extracts were tested on the isolated heart. Plasma extracts were adjusted to pH 6.8 prior to assay. Volumes not larger than 0.4 ml. were used for assay.

The number of experiments involved in each category may be seen in the legend of each figure.

FIGURE 2
Responses of isolated perfused guinea pig heart to:
† 1: 0.0004 µg. of norepinephrine
† 2: 5 µg. of metanephrine
† 3: 10 µg. of normetanephrine
† 4: 10 µg. of 3-methoxy-4-hydroxymandelic acid
† 5: 0.0008 µg. of norepinephrine
† 6: 10 µg. of metanephrine
† 7: 15 µg. of normetanephrine
† 8: 20 µg. of 3-methoxy-4-hydroxymandelic acid. Number of experiments in each group: 6.

for that category. Usually, a single isolated heart was used to test no more than twelve samples of extracts or of crude plasma. This varied, however, depending on how well the heart was responding and performing.

Results
The positive inotropic responses of the isolated perfused guinea pig heart to standard solutions of epinephrine, norepinephrine, metanephrine, normetanephrine, and 3-methoxy-4-hydroxymandelic acid are shown in figures 1 and 2. These figures illustrate the very high sensitivity of this preparation toward catecholamines as compared with the responses to the main catecholamine metabolites.

Table 1 shows the calculated plasma levels which result following epinephrine or norepinephrine infusion. Sample calculations for both crude plasma and plasma aluminum oxide responses are presented.

The effect of pretreatment with DCI on the responses of the isolated heart to epinephrine and norepinephrine, and to mixtures of both catecholamines is shown in figures 3 and 4. It may be noted that when the isolated heart is under influence of a 50-µg. dose of DCI, the response to epinephrine is completely blocked, while the heart continues to respond.

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Comparison of Plasma Levels of Epinephrine and Norepinephrine Following Constant Infusion of 1.5 \(\mu\)g/Kg./min.*

<table>
<thead>
<tr>
<th>Catecholamine Infused</th>
<th>Control plasma concentration (\mu)g./L.</th>
<th>Plasma concentration during infusion, (\mu)g./L. Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 min.</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>15 (&lt;) 0.5</td>
<td>Crude plasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\text{Al}<em>{2}O</em>{3}) extract</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>15 (&lt;) 0.5</td>
<td>Crude plasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\text{Al}<em>{2}O</em>{3}) extract</td>
</tr>
</tbody>
</table>

*Levels were determined based on responses of the isolated heart of the guinea pig to standards of known concentration and were compared with samples of plasma and of plasma aluminum oxide extracts. See sample calculations below.

Sample calculations:

**Crude Plasma**—If 0.2 cc. of crude plasma causes a response on the isolated heart equal to that caused by 0.0004 \(\mu\)g. norepinephrine or epinephrine, this would mean that 0.2 cc. of plasma contains 0.0004 \(\mu\)g. of the catecholamine. The catecholamine content per liter would be 5,000 times 0.0004 or 2 \(\mu\)g. per liter.

**Aluminum Oxide Extract**—A 10-cc. eluate from an aluminum oxide extract usually represents the catecholamines in 10 cc. of plasma. A 2-cc. quantity of this eluate is adjusted to pH 6.8. If 0.2 cc. of this quantity gives a response equal to 0.0004 \(\mu\)g. of standard catecholamines, the content in 1,000 cc. of plasma would be 0.0004 \(\times\) 5000 or 2 \(\mu\)g. Comparisons with other standards are treated similarly.

**FIGURE 3**

Responses of isolated perfused guinea pig heart to:

1: 0.1 \(\mu\)g. of epinephrine

2: 0.1 \(\mu\)g. of norepinephrine

I : control responses

II : after 30 \(\mu\)g. of DCI Single injection

III : after 50 \(\mu\)g. of DCI Single injection

Number of experiments in each group: 10.

to norepinephrine (fig. 3), thus permitting a differentiation of these catecholamines when administered in a mixture (fig. 4).

The responses of the isolated heart to crude plasma samples before, at various intervals during, and 30 minutes after the infusion of epinephrine are given in figure 5. It is of interest that samples taken 30 minutes after infusion had been stopped showed some positive inotropic effect. Figure 5 also shows that the responses to crude plasma samples are essentially the same as those to the extracts of the same samples. On the other hand, it may be seen that when the heart is treated with DCI, aliquots of the same samples which gave positive inotropic effects before DCI treatment gave either no positive inotropic effects or they gave negative inotropic effects.

Figure 6 shows the responses of the isolated perfused guinea pig heart to crude plasma samples and to plasma extracts following the infusion of norepinephrine.

**Discussion**

Our experimental results confirm and extend earlier observations showing the very high sensitivity and specificity of the isolated perfused guinea pig heart preparation as an assay procedure for epinephrine and norepinephrine. Also, it may be seen that the metabolites of epinephrine and norepinephrine are several hundred times less effective on the heart than the original substances. The argument is, therefore, not tenable that responses which we see in the infusion experiments are due to metabolites of epinephrine and norepinephrine.

Of special interest is the observation that 30 to 50 \(\mu\)g. of DCI completely block the re-
Responses of isolated perfused guinea pig heart, pretreated with 30 μg of DCI, to:
1: 0.002 μg of epinephrine
2: 0.002 μg of norepinephrine
3: mixture of 0.001 μg of epinephrine + 0.001 μg of norepinephrine
4: mixture of 0.0005 μg of epinephrine + 0.0012 μg of norepinephrine
5: mixture of 0.0004 μg of epinephrine + 0.0016 μg of norepinephrine.
Number of experiments in each group: 10.

Responses of isolated perfused guinea pig heart to 0.2-ml samples of crude plasma (I) and plasma extracts (II, III) before, during, and after intravenous infusion of 1.5 μg/Kg/min. of epinephrine, for 30 minutes, in dogs:
1: before infusion
2: after 10-minute infusion
3: after 20-minute infusion
4: after 30-minute infusion
5: 30 minutes after the end of infusion
I and II: before pretreatment of the isolated heart with DCI
III: after pretreatment of the isolated heart with 100 μg of DCI.

The experimental results further show that there is no essential difference in the pattern of response of the isolated guinea pig heart to crude plasma samples as compared with extracts of the same samples. The fact that
FIGURE 6
Responses of isolated perfused guinea pig heart to 0.4-ml samples of crude plasma (I) and plasma extracts (II) before, during, and after intravenous infusion of 1.5 µg/Kg/min. of norepinephrine, for 30 minutes, in dogs:

1. before infusion
2. after 10-minute infusion
3a. after 30-minute infusion
3b. after 20-minute infusion.

the crude plasma samples provoke a slightly more pronounced positive inotropic effect than the extracts may be attributed to the incomplete recovery of the catecholamines during the adsorption and elution procedures. Thus, the use of crude plasma samples in the estimation of catecholamine changes, following infusion or following drug administration that might result in increased plasma levels of catecholamines, may be considered a rapid and reasonably safe procedure. It should be emphasized, however, that for reliability of results, the control samples should show very little inotropic effect on the heart.

Pekkarinen and Lund, using physicochemical methods of estimation, reported that after intravenous injection of epinephrine the amine was completely eliminated from the blood within 10 minutes. In contrast to these findings, De Schaepdryver and Axelrod et al. were able to show that after intravenous infusion of C14-epinephrine and the injection of H3-epinephrine, respectively, significant amounts of epinephrine were present in the plasma for more than two hours. Our experimental results are in agreement with the observations of these authors.

Summary
Experiments have been described which show the very high sensitivity of the isolated perfused guinea pig heart preparation in the estimation of plasma levels of catecholamines after intravenous infusion in dogs. It was observed that crude plasma samples show the same pattern of positive inotropic response as extracts of these samples. By means of the isolated guinea pig heart method, the presence of circulating catecholamines could still be detected 30 minutes after the end of infusion.

The observation that dichloroisoproterenol may block the positive inotropic effect of epinephrine, while only slightly reducing the positive inotropic effect of norepinephrine, may be used to differentiate between these two catecholamines.

References
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