EFFECT OF DIBENAMINE (N-(2-CHLOROETHYL)DIBENZYL-AMINE) ON THE METABOLISM OF RADIOACTIVE EPINEPHRINE

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Dibenamine (N-(2-chloroethyl)dibenzylamine), a powerful adrenergic blocking agent, is known to modify the pharmacological effects of epinephrine and to reduce the toxicity (1). This laboratory has been studying the metabolism of epinephrine (2-4) and has shown the existence of at least five urinary metabolites. The problem of isolation and identification of these metabolites would be considerably simplified if Dibenamine-treated rats were employed, for this treatment permits a 2- to 3-fold increase in the dose of epinephrine tolerated, with a corresponding increase in the quantity of urinary metabolites. We have therefore made an investigation on the effect of Dibenamine on the pattern of urinary metabolites and on the rate of metabolism of epinephrine.

EXPERIMENTAL

Isotopic Compounds—Synthetic procedures have been published for \( \beta \)-C\( ^{14} \)-dl-epinephrine (5), methyl-C\( ^{14} \)-dl-epinephrine (4), and the \( l \) isomers of both types (4).

Effect of Dibenamine on Urinary Metabolites of Epinephrine—A chromatogram of urine from a rat given Dibenamine followed by \( \beta \)-C\( ^{14} \)-dl-epinephrine is shown in Fig. 1. This chromatogram conforms closely to the picture of the urinary metabolites of epinephrine which has gradually been formulated not only from chromatograms but also from differences in urinary excretion of C\( ^{14} \) after administration of methyl-labeled and \( \beta \)-labeled epinephrine, from studies of ether-soluble fractions, and from isotope dilution assays for unchanged epinephrine (2-4). Each peak will be discussed separately.

Peak 1 is due to a substance not found in chromatograms of urine of rats given methyl-labeled epinephrine. Therefore, the methyl carbon atom has been lost. It is believed to be due to one of the substances which are

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1 Obtained from Givaudan-Delawanna, Inc., New York, New York.
rendered ether-soluble by acid hydrolysis. Ether Fraction IV, which constitutes 5.3 per cent of the total radioactivity of this urine, or to the portion of Ether Fraction II extracted by sodium bicarbonate solution, which contributes 10.7 per cent of the total radioactivity.

Peak 2 is due to a substance which also occurs in chromatograms of urine of rats given methyl-labeled epinephrine. The methyl carbon atom is retained. Adrenochrome (6) is not intermediate in its formation (7).

Peak 3 does not occur in chromatograms of urine of rats given methyl-labeled epinephrine. It is probably the major urinary metabolite produced by demethylation of epinephrine effected by amine oxidase. The substance may be 3,4-dihydroxymandelic acid or a derivative of it.

Peak 4 is probably due to unchanged epinephrine. It occurs at the same position in chromatograms of urine of rats given methyl-labeled epinephrine and has an $R_F$ value approximately that of epinephrine. Isotope dilution assay of this urine showed that epinephrine contributed about 17 per cent of the total radioactivity.

Peak 5 is due to a substance also found in chromatograms of urine of rats given methyl-labeled epinephrine; hence it retains the methyl carbon. Adrenochrome is not an intermediate in its formation (7).

Peak 6 is not found in chromatograms of urine of rats given methyl-

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* Ether Fraction I is the fraction extractable by ether from acidified urine; Ether Fraction II is the fraction extractable by ether from acid-hydrolyzed urine from which Ether Fraction I had been removed previously; Ether Fraction III is the ether portion after extraction of Ether Fraction I with 1 per cent sodium bicarbonate solution; Ether Fraction IV is the ether portion after extraction of Ether Fraction II with 1 per cent sodium bicarbonate solution.
labeled epinephrine. It is probably the substance extractable with ether before hydrolysis, Ether Fraction I. The value of Ether Fraction I in this urine was 4.3 per cent.

We have previously presented a chromatogram of the urine of a rat given \(\beta\)-C\(^{14}\)-dl-epinephrine which is similar in most respects to this chromatogram (2). However, the present chromatogram is considerably sharper, owing to the increased dose of epinephrine permitted by the use of Dibenamine. Chromatograms of urine of rats given Dibenamine followed by methyl-C\(^{14}\)-dl-epinephrine do not differ significantly in any respect from our published chromatograms (4) of urine of rats given methyl-labeled epinephrine alone. These chromatograms show only three peaks, corresponding to Peaks 2, 4, and 5 of Fig. 1.

Effect of Dibenamine on Rate of Metabolism of Epinephrine—Male Swiss albino mice, 18 to 21 gm., were divided into two groups of nine each. One group was injected subcutaneously with 200 \(\gamma\) of Dibenamine hydrochloride in saline; the control group was injected with saline only. 30 minutes later each mouse was injected intraperitoneally with 20 \(\gamma\) of methyl-C\(^{14}\)-l-epinephrine. Exactly 20 minutes after injection each mouse was killed by a blow on the head and was immediately frozen by immersion in dry ice-acetone. For analysis each mouse was placed in warm water to thaw the skin, the skin removed, and the carcass homogenized (Waring blender) in 20 ml. of 0.1 \(N\) HCl containing 300 mg. of non-isotopic epinephrine carrier and ascorbic acid to prevent autoxidation. To the homogenate was added the cut up skin, followed by trichloroacetic acid, and the mixture was allowed to stand at room temperature for 30 minutes with frequent stirring. After filtration trichloroacetic acid was extracted from the filtrate with ether, and then the ether was removed with nitrogen admitted through a gas dispersal tube. Excess ammonium hydroxide was added and the gelatinous precipitate of metal hydroxides and phosphates removed immediately by filtration through Super-Cel. The filtrate was scratched to induce crystallization of epinephrine and let stand under nitrogen or hydrogen for several hours in the cold. The epinephrine recovered usually amounted to 80 to 150 mg. It was counted and then recrystallized by dissolving in 2 per cent acetic acid, adding Norit, filtering, and precipitating with ammonia. Constant radioactivity was always reached after one recrystallization.

Each sample was counted at (or corrected to) infinite thickness. The original solution injected into the mice was assayed before and after the series of injections by adding 20 \(\gamma\) of epinephrine to 300 mg. of carrier, reprecipitating, and counting at infinite thickness. Thus the per cent of

\*We have found that a level of 10 \(\gamma\) of Dibenamine hydrochloride per gm. of body weight permits a 2- to 3-fold increase in the dose of epinephrine tolerated by rats.
epinephrine in each mouse is simply the observed counts per minute at infinite thickness of the isolated epinephrine divided by the observed counts per minute at infinite thickness of the epinephrine from the assay of the original solution. All counting was done in flow counters having a background about 20 c.p.m. in plates of 4.5 cm. square. All observed counts were at least twice that of the background. The results are presented in Table I.

<table>
<thead>
<tr>
<th>Preliminary treatment (9 mice in each experiment)</th>
<th>Per cent C14 epinephrine unmetabolized 20 min. after intraperitoneal injection</th>
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</thead>
<tbody>
<tr>
<td>200 γ Dibenamine hydrochloride subcutaneously 30 min. before administration of epinephrine Control</td>
<td>18, 18, 19, 19, 21, 21, 21, 25, 29, average 21* 30, 30, 31, 32, 32, 33, 36, 53, 79, † average 35*</td>
</tr>
</tbody>
</table>

* The difference between the two averages is 14 per cent. Since control mice metabolized 65 per cent of the epinephrine in 20 minutes, the increased rate of metabolism in Dibenamine-treated mice is $14/65 = 22$ per cent.
† Omitted from the average. This high value may have been due to the injected epinephrine entering the intestine, where it would be slowly metabolized.

**DISCUSSION**

The results show that, under the experimental conditions used, Dibenamine accelerates the destruction of epinephrine in the mouse, producing an increase in rate of about 20 per cent. A possible explanation is that, under normal conditions, epinephrine is attached to certain "receptors" where it is temporarily removed from destruction by its metabolic enzymes. If, however, Dibenamine successfully competes for position on these "receptors," a portion of the epinephrine loses this protection and is metabolized more rapidly. Explanation of the increased destructive rate in terms of activation of (or removal of inhibition from) the metabolic enzymes of epinephrine seems less plausible, for this would necessitate postulation of an equal degree of activation of all the various enzyme systems involved in order to satisfy our observations that Dibenamine produces no change in the relative proportions of the urinary metabolites. The observed increase in rate of destruction of epinephrine, although significant, is insufficient to explain the greater tolerance of Dibenamine-treated animals to epinephrine.

The chromatogram, Fig. 1, reveals a pattern of urinary metabolites which is in accord with other data on epinephrine metabolites. Inspection of Fig. 1 indicates that roughly one-half of the epinephrine is metabolized
by loss of the methyl carbon atom, for Peaks 1, 3, and 6 from which the methyl carbon has been lost are approximately equal in magnitude to Peaks 2 and 5 which retain the methyl carbon. We have previously shown by other means that roughly one-half of injected l- or dl-epinephrine is metabolized by a route involving loss of the methyl carbon atom (3, 4).

In the urine sample used for the chromatogram of Fig. 1, the values of Ether Fractions I, II, III, and IV are 4.3, 16, 0, and 5.3 per cent, respectively. These values compare closely with values previously published from urine of rats given subcutaneously 3 / of C\textsuperscript{14} dl epinephrine per gm. of body weight: 5.6, 18, 0, and 7.0 per cent (3).

The use of dl-epinephrine in some of these experiments seems justified since it produces metabolic products similar to those from natural l-epinephrine (4). There is no evidence from this work that Dibenamine changes the number, nature, or relative abundance of the urinary metabolites of epinephrine.

**SUMMARY**

Dibenamine (N-(2-chloroethyl)dibenzylamine), an adrenergic blocking agent, produces no observable change in the number, nature, or relative abundance of the urinary metabolites of epinephrine. It does, however, cause a significant increase in the rate of destruction of l-epinephrine in the mouse.

**BIBLIOGRAPHY**

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