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METABOLIC PRODUCTS OF ADRENALINE (EPINEPHRINE) DURING LONG-TERM CONSTANT RATE INTRAVENOUS INFUSION IN THE HUMAN*

H. ALTON and McC. GOODALL

Departments of Physiology and Surgery, University of Texas Medical Branch, Galveston, Texas, U.S.A.

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Abstract—Four subjects were infused with 0·063 μg/kg/min of *dl*-adrenaline-2-^{14}C (10 μc) for 8 hr. Urine was collected at 2-hr intervals for 12 hr and then at the end of 18 and 24 hr. The urinary metabolites were separated and quantitated by combination column and filter paper chromatography. The radioactivity recovered in the urine reached a constant level 4 hr after the beginning of the infusion and continued at a constant level for 4 hr. The primary metabolites of adrenaline, i.e. metradrenaline and 3,4-dihydroxymandelic acid (DOMA), were recovered in constant amounts after 2 hr, whereas the secondary metabolites, 3-methoxy-4-hydroxymandelic acid (MOMA) and metadrenaline sulfate, were not recovered in constant amounts until after 4 hr. During this period of constant urinary radioactivity, the infusion rate was 1·25 μc/hr, DOMA was recovered in the urine at 0·018 μc/hr, free metadrenaline at 0·031 μc/hr, metradrenaline sulfate at 0·30 μc/hr and MOMA at 0·24 μc/hr. By extrapolating these results in terms of the normal endogenous rate of adrenaline secretion (860 μg/24 hr), it can be calculated from Table 1 that on the order of 100–300 μg/hr of MOMA normally occurs in the urine as the result of adrenaline metabolism. Since the normal urinary output of MOMA is 3–5 mg/24 hr, this then means that most of the MOMA is derived from endogenous noradrenaline.

WHEREAS the metabolism of adrenaline (epinephrine) in the human has been studied after short-term intravenous administration,[1–6] hitherto it has not been studied after a long-term constant rate infusion, which is more nearly like the normal release of adrenaline from the adrenal medulla. The quantity of adrenaline given in the previous short-term i.v. injection experiments,[1–6] calculated as the *l*-isomer, varied between 0·08 μg/kg/min and 0·37 μg/kg/min, which is considerably higher than the amount that is normally supplied endogenously by the adrenal medulla during resting conditions, i.e. approximately 0·01 μg/kg/min of *l*-adrenaline.[7] Therefore, the following experiments were so designed that the amount of exogenously infused adrenaline was maintained at a level that was slightly above the normal resting endogenous release of adrenaline. In order to simulate the normal quiescent state of adrenaline metabolism, it is necessary that a constant level of excretion be established among the various urinary metabolic products. Therefore, in these experiments the infusion of adrenaline was continued at a steady rate for a sufficiently long period to obtain this equilibrium.

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METHODS

Four normal healthy females between the ages of 18 and 25 yr were infused with a total of 10 μc (9·1 μmole) of *dl*-adrenaline-2-^{14}C-*d*-bitartrate.* The labeled adrenaline was mixed with 800 ml physiological saline and infused at a steady rate into the antecubital vein over a period of 8 hr. Each subject was catheterized and from the beginning of the infusion the urine was collected at 2-hr intervals for the first 12 hr and then at the end of 18 and 24 hr. In terms of body weight, the subjects received about 0·063 μg/kg/min of *dl*-adrenaline. Since the subjects were females and weighed between 50 and 60 kg, this would then mean that each subject received two to three times the amount of *l*-adrenaline normally supplied endogenously during rest, as determined by Cohen *et al.*[7]

The procedure for the quantitation and identification of the various urinary metabolites has been described in more detail previously.[6, 8] The separation and quantitation of the various urinary metabolites was accomplished by means of a combination of column fractionation and paper chromatography. An aliquot of urine was placed on a 1 × 5 cm column of Amberlite IRC-50.† The neutral and acidic metabolites passed through the column and were collected in the effluent. The basic metabolites, i.e. adrenaline, metadrenaline and an unknown, were eluted from the IRC-50 resin with 0·5 N acetic acid.

An aliquot of the eluate containing 500–1500 dpm was evaporated to approximately 1 ml and chromatographed for 24 hr on Whatman No. 1 paper; *n*-butanol saturated with 1 N HCl was used as the solvent. After drying, the paper was cut into 1-cm strips. Each strip was placed in a 20-ml counting vial filled with scintillation liquid and its radioactivity was measured with a Tri-Carb liquid scintillation spectrometer. Three radioactive peaks were obtained, corresponding to adrenaline, metadrenaline and an unknown compound. The percentage of radioactive adrenaline, metadrenaline and the unknown substance in each sample was calculated and, when interpreted in terms of the total radioactivity recovered in the eluate of the Amberlite column, gave information as to the total amount of radioactivity of each compound.

An aliquot of acidic and neutral metabolites (effluent) was placed on a 0·9 × 45 cm column of Dowex-1-X2‡ acetate ion-exchange resin. The column was placed on an automatic fraction collector and eluted with 75 ml distilled water. The column was then attached to an automatic gradient elution system consisting of four series-connected cylinders, each of which contained 275 ml of solution: the first contained distilled water; the second, 1·5 M ammonium acetate buffer (pH 4·8); the third, distilled water; and the fourth, 6 M ammonium acetate buffer (pH 4·8). During the elution the flow rate was maintained at a rate somewhat less than 0·5 ml/min; 5-ml fractions were collected. Throughout the course of the elution, alternate fractions were assayed for radioactivity in an automatic low background planchet counter. Fractions constituting a single radioactive peak were pooled and the radioactivity of the pooled samples was measured by liquid scintillation.

The recovery of the total radioactivity placed on the Dowex-1 column was 97 ± 6 per cent. The peaks containing specific free phenolic acids, such as 3-methoxy-4-

* *dl*-Adrenaline-2-^{14}C bitartrate salt; sp. act., 1·10 mc/m-mole from Nuclear-Chicago Corp., Des Plaines, Ill.

† Amberlite IRC-50 (IRP-64) from Rohm and Haas Company, Philadelphia, Pa.

‡ Dowex-1-X2, 200–400 mesh, chloride form, from Bio-Rad Laboratories, Richmond, Calif.

TABLE 1. EXCRETION PATTERN OF ADRENALINE AND ITS METABOLITES DURING AN 8-hr CONSTANT RATE INFUSION AND FOR 10, 12, 18 AND 24 hr POST-INFUSION*

Time interval of collection after beginning infusion	IRC-50 fractions			DOWEX-1 fractions											Neutral fraction	Per cent Recovery of infused dose
	Adr	Metadr	Unk	MOPEG and DOPEG	Metadr-SO_4 conjugate	Metadr conj. 2 (glucuronide)	PIB Unk	MOMA	DOMA	HVA and DOPAC	VA	MOPEG-SO_4	DOPEG-SO_4	Column residue	(Dowex-1 effluent)	
0–2 hr	13·5 ± 3·5	7·1 ± 1·4	1·0 ± 0·8	1·2 ± 0·1	17·1 ± 2·4	2·0 ± 1·0	1·9 ± 0·2	36·1 ± 1·6	2·1 ± 0·4	2·1 ± 0·5	1·1 ± 0·6	3·8 ± 0·6	IA	1·5 ± 0·6	2·4 ± 0·6	5·9 ± 0·9
2–4 hr	10·0 ± 1·8	5·5 ± 1·3	1·0 ± 0·3	1·8 ± 0·4	25·3 ± 2·7	1·3 ± 0·5	3·4 ± 0·7	30·7 ± 2·4	2·8 ± 0·6	2·3 ± 0·8	1·4 ± 0·5	3·3 ± 0·2	IA	1·4 ± 0·3	3·0 ± 0·7	12·5 ± 2·8
4–6 hr	6·4 ± 0·7	3·7 ± 0·7	0·8 ± 0·3	1·5 ± 0·4	32·8 ± 1·3	1·2 ± 0·3	3·7 ± 0·9	29·0 ± 2·3	2·2 ± 0·6	2·8 ± 0·9	1·2 ± 0·5	3·3 ± 0·8	IA	1·3 ± 0·3	3·5 ± 0·6	17·1 ± 0·7
6–8 hr	6·0 ± 1·0	3·5 ± 0·8	0·8 ± 0·3	1·8 ± 0·4	35·6 ± 3·2	1·7 ± 0·3	4·2 ± 0·8	28·2 ± 3·1	2·0 ± 0·3	2·7 ± 0·4	1·5 ± 0·8	3·5 ± 0·9	IA	2·2 ± 0·2	3·8 ± 0·6	17·6 ± 0·2
8–10 hr	0·8 ± 0·2	1·5 ± 0·4	0·5 ± 0·1	1·9 ± 0·3	44·8 ± 2·1	1·7 ± 0·5	4·8 ± 1·0	21·4 ± 1·8	1·2 ± 0·4	2·5 ± 0·8	1·5 ± 0·2	3·3 ± 0·9	1·7 ± 0·6	1·8 ± 0·2	5·4 ± 0·7	13·7 ± 0·4
10–12 hr	IA	0·8 ± 0·4	IA	1·9 ± 0·5	53·0 ± 1·5	1·6 ± 0·5	4·7 ± 2·0	16·4 ± 0·7	1·2 ± 0·7	2·0 ± 0·4	1·2 ± 0·5	3·8 ± 0·6	1·2 ± 0·5	2·5 ± 0·8	6·2 ± 0·9	7·6 ± 1·1
12–18 hr	IA	IA	IA	1·7 ± 0·5	56·3 ± 2·1	IA	3·6 ± 2·0	16·2 ± 0·9	IA	1·8 ± 0·6	IA	3·4 ± 0·6	1·2 ± 0·6	2·2 ± 0·5	5·1 ± 0·8	11·6 ± 0·7
18–24 hr	IA	IA	IA	IA	55·6 ± 2·0	IA	1·7 ± 1·0	19·8 ± 0·8	IA	IA	IA	4·3 ± 0·7	IA	2·0 ± 0·4	4·8 ± 2·0	5·2 ± 0·7
Total 24-hr recovery	4·5 ± 1·2	3·0 ± 0·5	0·7 ± 0·2	1·7 ± 0·2	39·4 ± 2·1	1·4 ± 0·3	3·8 ± 0·9	24·7 ± 2·0	1·8 ± 0·2	2·5 ± 0·6	1·3 ± 0·6	3·4 ± 0·6	IA	1·9 ± 0·2	4·2 ± 0·8	91·2 ± 1·3

* Results are expressed as per cent of recovered radioactivity ± S.D. Adr, adrenaline; Metadr, metadrenaline; Unk, unknown; MOPEG and DOPEG, 3-methoxy-4-hydroxyphenylglycol and 3,4-dihydroxyphenylglycol; Metadr-SO_4 conjugate, metadrenaline sulfate; Metadr conj. 2, metadrenaline glucuronide; MOMA, 3-methoxy-4-hydroxymandelic acid; DOMA, 3,4-dihydroxymandelic acid; HVA and DOPAC, 3-methoxy-4-hydroxyphenylacetic acid and 3,4-dihydroxyphenylacetic acid; VA, vanillic acid; MOPEG-SO_4, 3-methoxy-4-hydroxyphenylglycol sulfate; DOPEG-SO_4, 3,4-dihydroxyphenylglycol sulfate; IA, insignificant amount.

B.P.—*facing page 2164*

hydroxymandelic acid (MOMA), 3-methoxy-4-hydroxyphenylacetic acid (HVA), 3,4-dihydroxymandelic acid (DOMA), vanillic acid (VA), and 3,4-dihydroxyphenylacetic acid (DOPAC), were confirmed by paper chromatography of each peak in three solvent systems: *n*-butanol–N-acetic acid–H_2O (4:1:1); benzene–proprionic acid–H_2O (8:2:2); and isopropanol–5% NH_3 (8:2). In order to chromatograph these peaks, it was necessary to first remove the ammonium acetate. The conjugates of 3,methoxy-4-hydroxyphenylclycol (MOPEG) and 3,4-Dihydroxyphenylclycol (DOPEG) were identified by refluxing the ammonium acetate free peak in 3 N H_2SO_4 followed by extraction into ether at pH 6·5 and chromatography of the resulting compound with appropriate carrier compounds in the solvent systems described above.

RESULTS

The recovery of the infused radioactivity is shown in Table 1. During the 4–8 hr period, the urinary radioactivity remained essentially constant, indicating that a constant level had been established between infused and excreted (urinary) radioactivity. During the 4–8 hr period, 5 μc was infused and 3·4 μc was recovered or, that is, 0·85 μc/hr was recovered during this period. By extrapolating these results, it would appear that for a constant infusion rate of adrenaline, whether endogenous or exogenous, of 37·8 μg/kg/hr, as used in these experiments, about 25·7 μg/hr could be expected to be returned in the urine as adrenaline and its metabolites. At the end of 8 hr, 52 per cent of the infused radioactivity had been recovered, 66 per cent at the end of 10 hr, and by 24 hr 91 per cent of the infused radioactivity was returned.

It may be seen from Table 2 that a distinct difference exists in the time required to establish a constant level among the various urinary metabolites. The primary metabolites of adrenaline, metadrenaline (3-*O*-methyladrenaline) and DOMA, appeared in constant amounts by the end of 2 hr. From 2–8 hr, adrenaline was recovered at a constant rate of 0·052 μc/hr representing 4 per cent of the infused dose of 1·25 μc/hr, metadrenaline at 0·031 μc/hr representing 2·5 per cent and DOMA at 0·018 μc/hr representing 1·4 per cent.

The major secondary metabolites, MOMA and metradrenaline sulfate (3-*O*-methyladrenaline-4-*O*-sulfate) conjugate, were not obtained in constant amounts until during the 4–8 hr period. Metadrenaline sulfate, which represents the largest of the urinary metabolites in this period, was recovered at 0·3 μc/hr representing 24 per cent, and MOMA, the second largest metabolite, was recovered at 0·24 μc/hr representing 19 per cent of the infused dose of 1·25 μc/hr. The remainder of the radioactivity, 0·18 μc/hr recovered during the 4–8 hr period, was distributed among several minor metabolites as indicated in Table 1.

The calculated normal endogenous release rate of adrenaline is about 0·01 μg/kg/min,[7] corresponding to 860 μg/24 hr. In the present study, the amount of radioactivity recovered during the 6–8 hr infusion-collection period was 1·76 μc, which represented approximately 70 per cent of the 2·5 μc infused during this period. Extrapolating these results in terms of endogenous adrenaline, this would mean that approximately 600 μg/24 hr (860 μg/24 hr $\times$ 70 per cent) of adrenaline and its metabolites would appear in the urine (see Table 1, 6–8 hr period of collection). Based on the results in Table 1 in the 6–8 hr period, it may be calculated that 36 μg/24 hr (600 $\times$ 6·0 per cent) of adrenaline, 22 μg/24 hr of free metadrenaline, 12 μg/24 hr of DOMA, 210 μg/24 hr of conjugated metadrenaline, i.e. metadrenaline sulfate, and 165 μg/24 hr of MOMA

TABLE 2. RECOVERY OF ADRENALINE, METADRENALINE, METADRENALINE SULFATE CONJUGATE, MOMA AND DOMA*

Time interval of collection after beginning infusion	IRC-50 fractions		Dowex-1 fractions			Recovery of the infused dose
	Adrenaline	Metadrenaline	Metadrenaline-SO_4	MOMA	DOMA	
0–2 hr	0·080 ± 0·032	0·042 ± 0·014	0·101 ± 0·029	0·213 ± 0·041	0·013 ± 0·004	0·59 ± 0·09
2–4 hr	0·120 ± 0·048	0·068 ± 0·031	0·314 ± 0·103	0·381 ± 0·115	0·035 ± 0·015	1·25 ± 0·28
4–6 hr	0·108 ± 0·016	0·062 ± 0·014	0·561 ± 0·045	0·487 ± 0·058	0·037 ± 0·011	1·71 ± 0·07
6–8 hr	0·104 ± 0·018	0·061 ± 0·015	0·616 ± 0·062	0·488 ± 0·059	0·035 ± 0·006	1·76 ± 0·02
8–10 hr	0·011 ± 0·003	0·021 ± 0·006	0·614 ± 0·047	0·293 ± 0·033	0·016 ± 0·006	1·37 ± 0·04
10–12 hr		0·006 ± 0·004	0·403 ± 0·069	0·125 ± 0·023	0·009 ± 0·006	0·76 ± 0·11
12–18 hr†			0·217 ± 0·021 (0·653 ± 0·063)	0·063 ± 0·007 (0·188 ± 0·021)		0·38 ± 0·02 (1·16 ± 0·07)
18–24 hr†			0·096 ± 0·016 (0·289 ± 0·048)	0·034 ± 0·006 (0·103 ± 0·018)		0·17 ± 0·02 (0·52 ± 0·07)
Total 24-hr recovery	0·423 ± 0·118	0·260 ± 0·047	3·551 ± 0·237	2·278 ± 0·214	0·145 ± 0·018	9·12 ± 0·13

* Results are expressed in terms of μc recovered after an infusion of 10 μc *dl*-adrenaline-2-^{14}C over an 8-hr period. Calculated from the data in Table 1.
† Calculated on the basis of μc/2 hr; figures in parentheses represent total amount recovered.

are obtained from endogenous adrenaline. These results compare favorably with the findings of other investigators who found that adult males excrete 5–25 μg/24 hr of adrenaline[9, 10] and 100–300 μg/24 hr of metadrenaline (free and conjugated).[11, 12]

DISCUSSION

From the results in Table 1, it may be seen that a minimum of 4 hr is required to obtain constant urinary radioactivity. However, it has been demonstrated that steady state plasma concentration of adrenaline may be obtained during a constant rate infusion, such as that employed in this experiment, within 5 to 10 min.[7] In these experiments, however, the establishment of constant urinary radioactivity appears to be primarily dependent upon the rate of appearance of the major secondary metabolites of adrenaline, i.e. metadrenaline sulfate and MOMA (see Table 2). Adrenaline and its primary metabolites, i.e. metadrenaline and DOMA, appear in the urine at a constant level by the end of 2 hr and remain constant for 6 hr (2–8 hr), indicating that these compounds are rapidly formed (see Table 2). At least one of these, metadrenaline, is largely returned to the circulation and transported to other tissues to be bound or further metabolized. The fact that plasma concentrations of metadrenaline during short-term infusions of adrenaline are 2–3 times the plasma concentration of adrenaline[13] certainly implies that the incorporation of circulating adrenaline and the formation and release of metadrenaline at the cellular level is a very rapid process. DOMA has not been found in any significant quantities in the plasma during adrenaline infusions,[13] but this is due to the rapid conversion of DOMA to MOMA and other metabolites, i.e. 3,4-dihydroxybenzoic acid, 3,4-dihydroxyphenylacetic acid and vanillic acid.[14]

In earlier experiments on the metabolism of intravenously administered adrenaline. the recovery of the infused radioacitivity was considerably lower, i.e. 73–77 per cent in 24 hr,[1, 2, 6] than the recovery of 91·2 ± 1·3 per cent in 24 hr in these experiments. The reason for this is not entirely clear, since the differences in the distribution of adrenaline throughout the various tissues have not been investigated after a long-term infusion, and too, there is probably a difference in distribution between a long-term infusion and a transient increase of circulating adrenaline. However, when physiological doses of adrenaline are infused over long periods, there appears to be reflex suppression of the endogenous release of adrenaline. Considering the rapidity at which intravenously administered adrenaline is metabolized by COMT (catechol-*O*-methyltransferase) and MAO (monoamine oxidase), as evidenced by the rapid establishment of constant urinary levels of metadrenaline and DOMA (Table 2) and the high plasma levels of metadrenaline observed during an intravenous infusion of adrenaline,[13] it would be expected that a rapid turnover of the infused adrenaline and its primary metabolites, i.e. metadrenaline and DOMA, would occur. The secondary metabolites, i.e. metadrenaline sulfate and MOMA, are recovered in the urine at a constant level shortly after the primary metabolites (see Tables 1 and 2).

About 67 per cent of an i.v. infused dose of adrenaline is initially metabolized by COMT, while about 27 per cent is initially metabolized by MAO.[4, 6, 15] A somewhat similar situation exists when noradrenaline is intravenously infused,[8] but the relative amounts of noradrenaline that are initially metabolized by COMT or MAO have not been strictly determined. Table 3 shows a comparison of the major metabolites that were obtained after the intravenous infusion of adrenaline, noradrenaline or

normetadrenaline. It may be noted that, after the noradrenaline infusion, normetadrenaline (free and conjugated) represented 15·7 ± 0·3 per cent of the recovered radioactivity and MOMA represented 31·9 ± 1·5 per cent. After a 1-min infusion of adrenaline, metadrenaline and its conjugate accounted for 37·5 ± 2·8per cent and MOMA for 30·2 ± 5·3 per cent of the recovered radioactivity. In the present study

TABLE 3. RECOVERY OF SOME OF THE MAJOR METABOLITES OF INTRAVENOUSLY INFUSED ADRENALINE, NORADRENALINE AND NORMETADRENALINE*

Compound infused	Recovery of infused ^{14}C	Metadrenaline (free and conjugated)	Normetadrenaline (free and conjugated)	MOMA	DOMA
Adrenaline-2-$^{14}C^{6}$ (1-min infusion)	77·2 ± 7·6	37·5 ± 2·8		30·2 ± 5·3	1·6 ± 0·7
Noradrenaline-2-$^{14}C^{8}$ (1-min infusion)	81·7 ± 1·5		15·7 ± 0·3	31·9 ± 1·5	1·6 ± 0·6
Normetadrenaline-1-$^{14}C^{16}$ (1-min infusion)	79·8		36·3	38·3	None
Adrenaline-2-^{14}C (present study; 6–8 hr collection period or period of constant urinary radioactivity)	72·8 ± 3·8	39·1 ± 4·0		28·2 ± 3·1	2·0 ± 0·3

* Results are expressed in terms of per cent of the radioactivity recovered in 24 hr. MOMA, 3-methoxy-4-hydroxymandelic acid; DOMA· 3,4-dihydroxymandelic acid.

(Table 1) it may be seen that, of the radioactivity recovered during the 6–8 hr period, 39·1 ± 4·0 per cent was accounted for as metradrenaline and its conjugate and 28·2 ± 3·1 per cent as MOMA. Therefore, it appears that there is no significant difference between the metabolism of large doses of rapidly injected adrenaline representing transient stressful conditions to the body and the metabolism of small physiological doses representing the normal quiescent state of endogenous adrenaline release.

When one reviews the results of Tables 2 and 3 in terms of precursor–product relationship, it becomes apparent that both circulating adrenaline and noradrenaline contribute to the formation of MOMA, but they differ greatly in the extent of their contributions. Whereas 37·5 and 39·1 per cent of the recovered radioactivity from infused adrenaline is recovered as metadrenaline (free and conjugated), only 15·7 per cent of the infused noradrenaline is recovered as normetadrenaline (free and conjugated). Nevertheless, the amount of recovered radioactivity in terms of MOMA is nearly the same, thereby indicating that a considerably larger amount of circulating noradrenaline than of adrenaline must be initially metabolized via a route other than that of *O*-methylation. From our results, it was calculated that approximately 0·16 mg/24 hr of MOMA could be expected from the metabolism of endogenous circulating adrenaline. Recent evaluations place the normal urinary excretion of MOMA at 3·1 mg/24 hr (range, 2·3–11·1 mg/24 hr)[17] and 5·1 mg/24 hr (range, 2·7–7·6 mg/24 hr).[18] Therefore, MOMA derived from the metabolism of adrenaline, whether exogenous or endogenous, normally contributes relatively little to the total urinary MOMA, implying that the remainder must, in large part, be derived from the metabolism of endogenous noradrenaline via DOMA.

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