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O-Methylation, the principal pathway for the metabolism of epinephrine and norepinephrine in the rat

Previous studies have shown that epinephrine and norepinephrine are methylated on the 3-hydroxy position *in vitro* and *in vivo*[1]. Estimation of the extent to which O-methylation occurs in the intact animal was made possible by the development of a method for the determination of meta-O-methylepinephrine (metanephrine) and meta-O-methyl-norepinephrine (normetanephrine) in urine.

Metanephrine or normetanephrine was isolated from the urine at pH 10 by gently shaking for 20 min with 20 volumes ethylene dichloride* containing 2% isoamyl alcohol*. After the separation of the 2 phases by centrifugation, the aqueous layer was removed by aspiration and an aliquot of the organic phase was shaken with 1/10 volume 0.1 *N* HCl. The lower layer was removed by aspiration and the acid extract was washed twice with 10 volumes isoamyl alcohol to remove interfering substances. The acid extract was transferred to a quartz cuvette and metanephrine or normetanephrine was determined by measuring the fluorescence in an Aminco-Bowman fluorospectrophotometer at 335 mμ after activation at 283 mμ.

In order to correct for the partition of the methylated metabolites of the catechol amines in the two-phase system described above and the quenching of fluorescence that occurs occasionally, a known amount of synthetic metanephrine or normetanephrine was added to the urine to be assayed and carried through the procedure.

Glucuronides of metanephrine and normetanephrine were hydrolyzed by incubation of 1 ml of urine with 2,000 units of bacterial β-glucuronidase for 3 h at 37°.

After the administration of L-epinephrine or L-norepinephrine to rats, the methylated metabolites isolated from the urine by the procedure described above had the same R_F values, partition coefficients, and fluorescent spectra as authentic samples of metanephrine or normetanephrine[2].

TABLE I

O-METHYLATION OF EPINEPHRINE IN THE RAT

Each rat received 10 μg/g dibenamine hydrochloride intraperitoneally. After 30 min each rat was given a total of 5 μmoles of epinephrine or metanephrine intraperitoneally. The compounds were administered in 5 divided doses every hour for 5 h. Iproniazide (200 μg/g) was given intraperitoneally 2 h before the injection of the amines. Six rats were used in each experiment.

Compounds administered	*Metanephrine excreted* μmoles	*Metanephrine glucuronide excreted* μmoles	*Total excreted* %
L-Epinephrine (5 μmoles)	0.14	1.08	25
D, L-Metanephrine (5 μmoles)	0.42	1.31	35
L-Epinephrine (5 μmoles) and iproniazide	0.18	2.55	55
D, L-Metanephrine (5 μmoles) and iproniazide	0.50	3.00	70
D-Epinephrine (5 μmoles)	0.06	0.86	18
L-Epinephrine (3 μmoles) §	0.05	0.47	18

§ This group of rats did not receive dibenamine.

* All solvents, reagent grade, were washed successively with 1/5 volume 1 *N* NaOH, 1 *N* HCl and twice with distilled water.

L-Epinephrine bitartrate was administered intraperitoneally to rats, and the urine was collected for 24 h. Prior to the injection of the catechol amine, each rat received 10 mg/kg of dibenamine hydrochloride to reduce the toxic action of L-epinephrine. Urine was assayed for metanephrine before and after treatment with β-glucuronidase. To correct for fluorescing material, normally present in urine, "blank" urine was collected for 24 h prior to the injection of epinephrine and assayed as described above. From the results shown in Table I, about 21 % of the administered L-epinephrine was excreted as metanephrine glucuronide and about 4 % as the free methoxy metabolite. Essentially similar results were obtained after the administration of D-epinephrine bitartrate. Dibenamine did not appreciably affect the pattern of excretion of the methylated derivatives.

Further to delineate the metabolism of epinephrine, the excretion of metanephrine was examined after the administration of the latter compound (Table I). About one-third (35 %) of the administered metanephrine was excreted both in the unchanged form as well as a glucuronic acid conjugate. After the administration of epinephrine a total of 25 % metanephrine (free and conjugated) was found in the urine. This value represents about one-third of the epinephrine that underwent O-methylation. Accordingly, it may be inferred that the total amount of metanephrine formed after the administration of epinephrine was about 70 %.

When rats were pretreated with iproniazid, a monoamine oxidase inhibitor, the excretion of metanephrine was increased twofold (Table I). This observation suggests that monoamine oxidase deaminates considerable amounts of metanephrine, presumably to 3-methoxy-4-hydroxymandelic acid. This possibility seems likely in view of the observation of ARMSTRONG AND MCMILLAN that a major metabolic product of epinephrine is 3-methoxy-4-hydroxymandelic acid[3].

After the administration of L-norepinephrine to rats, a similar metabolic pattern was found (Table II).

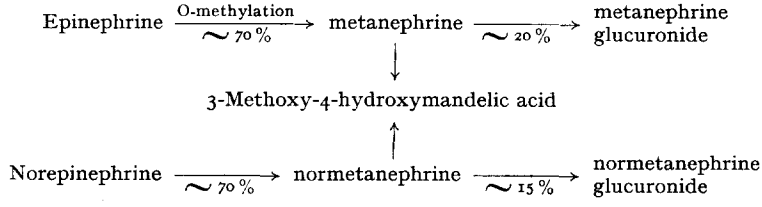
TABLE II

O-METHYLATION OF NOREPINEPHRINE IN THE RAT

Rats were treated in the same manner as described in Table I.

Compound administered	*Normetanephrine excreted* μmoles	*Normetanephrine glucuronide excreted* μmoles	*Total excreted* %
L-Norepinephrine (5 μmoles)	0.15	0.70	17
D, L-Normetanephrine (5 μmoles)	0.30	0.95	25
L-Norepinephrine (5 μmoles) and iproniazid	0.15	1.35	30
D, L-Normetanephrine (5 μmoles) and iproniazid	0.52	1.87	48

These results suggest the following scheme for the metabolism of epinephrine and norepinephrine in the rat:



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