Conversion of Norepinephrine to Epinephrine in Canine Testes[1] (34972)

DAVID H. MOORE AND KRISTEN B. EIK-NES
(Sponsored by Fred S. Grodins)

Division of Biochemistry and Physiology of Reproduction and Department of Physiology, University of Southern California, Los Angeles, California 90033

In 1968 Eliasson and Risley (1) reported on levels of the catecholamines, norepinephrine, and epinephrine in the testis, epididymis, and vas deferens of the cat, rabbit, guinea pig, and rat. Recently, Eik-Nes (2) observed that when isoproterenol, epinephrine, or norepinephrine was administered via the spermatic artery of the dog, secretion and production of testosterone increased. Norepinephrine was a weaker stimulant than epinephrine and exhibited its maximal effect on testosterone secretion at a slower rate than epinephrine. This difference may possibly reflect a gradual conversion of norepinephrine to epinephrine in the dog testis. The enzyme phenethanolamine *N*-methyl transferase catalyzes the conversion of norepinephrine to epinephrine (3), but its presence in vertebrate testes has not been reported. Axelrod and Goldzieher (4) demonstrated, however, that human testes contain transmethylation activity as shown by the conversion of 2-hydroxy steroids to their 2-methoxy analogs.

The purpose of this communication is to show that canine testes convert norepinephrine to epinephrine *in vivo*.

Experimental Procedure. Testes were infused via the spermatic arteries as described by our laboratories (2). The constant flow of arterial blood in this preparation (animal preparation no. 2, see Ref. 2) was 3.86 ml per min. Tritiated norepinephrine (DL-norepinephrine-7-^{3}H, specific activity 5 Ci/mmole, obtained from New England Nuclear) was dissolved in 0.9% sodium chloride solution and added to the arterial blood in the spermatic artery at a constant rate of 0.38 ml/min. When used, human chorionic gonadotropin, (HCG, obtained from Ayerst Laboratories) was added to the 0.9% sodium chloride solution containing tritiated norepinephrine.

Spermatic venous blood from infused testes was collected in 15-min samples over the 90-min infusion period and centrifuged at 1000*g* for 30 min. Plasma fractions were adjusted to 0.4 *N* with 4 *N* perchloric acid and refrigerated for 18 hr. Infused testes

TABLE I. Recrystallization of Epinephrine-^{3}H from Spermatic Venous Blood of Dogs Infused with Norepinephrine-^{3}H via the Spermatic Artery.[a]

Exp.	Recrystallization no.[b]	Specific radioactivity (dpm/mg)	% Deviation of specific radioactivity for recrystallizations 4–6
I	1	3490	
	2	3250	
	3	3000	
	4	2520	0.1
	5	2530	0.7
	6	2500	0.8
II	1	1540	
	2	1450	
	3	1340	
	4	1140	3.0
	5	1100	0.8
	6	1080	2.2

[a] Spermatic venous blood was processed as outlined. After paper chromatography in *n*-butanol: acetic acid:water (4:1:1), material behaving chromatographically like epinephrine was recrystallized six times from methanol.

[b] Solvent: methanol.

[1] This work was in part supported by Research Grant HD 04195-01 and by Training Grant CA 05216-01 from National Institutes of Health, Bethesda, Maryland.

were weighed and homogenized (Waring Blendor) in 60 ml 0.4 *N* perchloric acid. The homogenate was refrigerated for 18 hr.

Deproteinized plasma and tissue samples were centrifuged (4°) at 30,000*g* for 30 min, followed by addition of 500,000 dpm and 250,000 dpm DL-epinephrine-7-^{14}C (specific activity 45 mCi/mmole, obtained from New England Nuclear) to the plasma and tissue supernatant fractions, respectively, for the purpose of determining epinephrine recovery. After addition of 10 mg ascorbic acid and 250 mg EDTA to each supernatant fraction, the pH was adjusted to 8.4–8.5 with 3 *N* NaOH and the resulting solution poured on to a column of alumina (1 g), prepared according to the method of Anton and Sayre (5). The effluent was allowed to flow at a rate of 3 ml/min and was discarded. The column was then washed with 10 ml water and the catecholamines were eluted with 10 ml 0.4 *N* acetic acid.

The final eluate was evaporated under vacuum at 30°, the residue dissolved in a minimum volume of 0.2 *N* acetic acid (in methanol) and applied to 4 × 50 cm paper strips (Whatman No. 1) previously washed with 0.4 *N* acetic acid and sprayed with aqueous ascorbic acid solution (50 mg/100 ml). Authentic epinephrine and norepinephrine were chromatographed on reference chromatograms. The paper strips were developed in *n*-butanol:acetic acid:water (4:1:1) for 30 hr, dried under nitrogen, and scanned for radioactivity with a Packard radiochromatogram scanner. Areas of sample chromatograms exhibiting chromatographic behavior like epinephrine and norepinephrine were eluted with 0.4 *N* acetic acid.

To each eluate was added 24 mg DL-epineph-

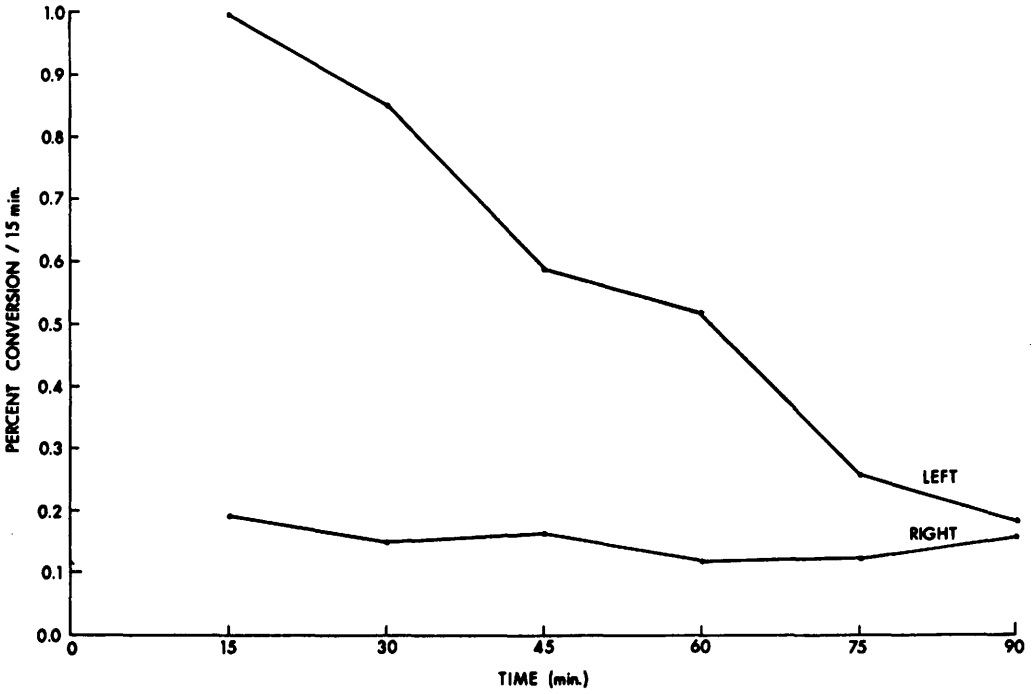

FIG. 1. Rates of conversion (%/15 min) of norepinephrine-^{3}H to epinephrine-^{3}H by left and by right testis of same dog. Animal preparation used is described in Ref. 2 (preparation II). During time 0–90 min left testis was infused with 20 IU HCG/min and 5,117,360 dpm norepinephrine-^{3}H while right testis was infused with 5,285,950 dpm norepinephrine-^{3}H/min. The spermatic venous plasma was processed as described, and after the fourth recyrstallization (from methanol) ^{14}C and ^{3}H were determined in each sample. Right testis: 60 dpm epinephrine/g tissue. Left testis: 720 dpm epinephrine/g tissue.

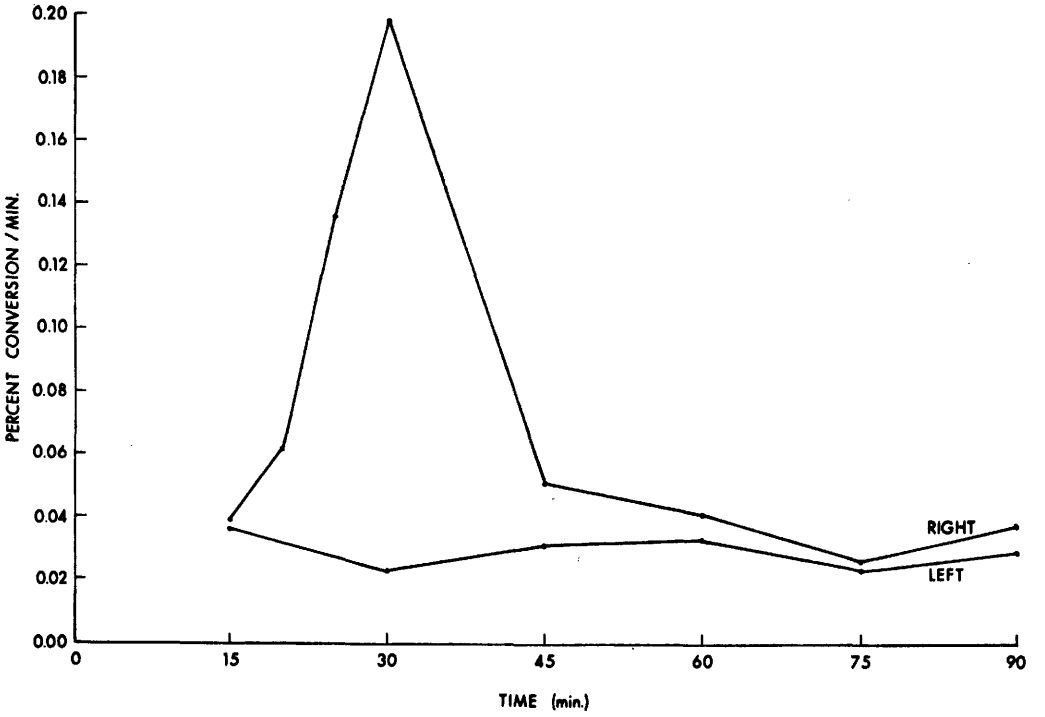

FIG. 2. Rates of conversion (%/min) of norepinephrine-^{3}H to epinephrine-^{3}H by left and by right testis of same dog. Animal preparation used is described in Ref. 2 (preparation II). During time 0–90 min right testis was infused with 4,439,100 dpm norepinephrine-^{3}H/min and left testis was infused with 4,519,100 dpm norepinephrine-^{3}H/min. During time 15–90 min right testis was also infused with 20 IU HCG/min. Details for processing plasma samples are given in legend of Fig. 1. Right testis: 240 dpm epinephrine/g tissue. Left testis: 210 dpm epinephrine/g tissue.

rine:HCl (K & K Laboratories; equivalent to 20 mg free amine), and free epinephrine in this mixture was precipitated by addition of cold aqueous NH_4OH solution (58%). The precipitate was washed twice with cold methanol and recrystallized six times from boiling methanol. Aliquots from each recrystallization were weighed on a Cahn microbalance and counted by liquid scintillation; specific radioactivity was constant over the last three recrystallizations. Recrystallized samples were subjected to thin-layer chromatography on glass plates coated with buffered silica gel (6). These plates were developed in phenol:acetic acid:water (8:1:3) (7). Authentic epinephrine (R_F .65) and norepinephrine (R_F .45) were chromatographed on separate lanes and tritium radioactivity in sample epinephrine behaved chromatographically like authentic epinephrine.

Results and Discussion. Blood plasma from testes infused with norepinephrine-^{3}H via the spermatic artery was processed as described. It is apparent that this plasma contained epinephrine-^{3}H since the material behaving chromatographically like epinephrine could be recrystallized to contant specific radioactivity (Table I). One should realize, however, that only after the first three recrystallizations did the biosynthesized epinephrine retain constant specific radioactivity. No significant amount of epinephrine-^{3}H could be estimated by this technique when norepinephrine-^{3}H was exposed to dog blood at 25° for 90 min.

In Figs. 1 and 2 are depicted the rates of conversion of norepinephrine-^{3}H to epinephrine-^{3}H after infusion of norepinephrine-^{3}H via the spermatic artery of the anesthetized dog. Compared to conversion rates under control conditions, HCG infusion via the spermatic artery appeared to increase conversion

of norepinephrine-^{3}H to epinephrine-^{3}H. This stimulation by HCG lasts only for about 30 min (Figs. 1 and 2). It is, however, possible that the conversion of norepinephrine-^{3}H to epinephrine-^{3}H is continuing to increase with time of HCG administration, but that epinephrine-^{3}H is diluted by endogenous epinephrine biosynthesized from endogenous norepinephrine. Compared to control testes, infusion of HCG via the spermatic artery also resulted in higher concentrations of epinephrine-^{3}H in testicular tissue.

A lower concentration of epinephrine (<0.05 μg/g) than of norepinephrine (0.02–0.2 μg/g) has been reported by Eliasson and Risley (1) in testes of various vertebrate species. The results obtained by us on percentage of conversion of norepinephrine-^{3}H to epinephrine-^{3}H (0.2–1.0%) in canine testes are, therefore, of significant nature. Since administration of HCG increased concentration of epinephrine-^{3}H both in spermatic venous blood as well as in testicular tissue, HCG may promote synthesis *de novo* of epinephrine from norepinephrine, either via increased production of the enzyme phenethanolamine *N*-methyl transferase or via increased production of necessary cofactors for this enzymic activity. Coupland (8) and Pohercky and Wurtman (9) demonstrated that glucocorticoids cause an induction or stimulation of this enzyme in extra-adrenal chromaffin tissue. Since it is known that HCG promotes synthesis and secretion of testosterone in the testis (10), experiments must be conducted on the effect of testosterone, or its intermediates from Δ^5-pregnenolone, on the biotransformation of norepinephrine to epinephrine in the male gonad. Currently we do not know if this production of epinephrine takes place in the Leydig cells, in the germinal epithelium, or in both cell types.

1. Eliasson, R., and Risley, P. L., Acta Physiol. Scand. **73**, 311 (1968).

2. Eik-Nes, K. B., Amer. J. Physiol. **217**, 1764 (1969).

3. Axelrod, J., J. Biol. Chem. **237**, 1657 (1962).

4. Axelrod, L. R., and Goldzieher, J. W., Endocrinology **70**, 943 (1962).

5. Anton, A. H., and Sayre, D. F., J. Pharmacol. Exp. Ther. **138**, 360 (1962).

6. Teichert, K., Mutschler, E., and Rochelmeyer, H., Deut. Apoth. Z. **100**, 283 (1960).

7. Choulis, N. H., J. Pharm. Sci. **56**, 196 (1967).

8. Coupland, R. E., J. Endocrinol. **41**, 487 (1968).

9. Pohorecky, L. A., and Wurtman, R. J., Nature (London) **219**, 392 (1968).

10. Eik-Nes, K. B., *in* "The Androgens of the Testis" (K. B. Eik-Nes, ed.), pp. 1–40. Dekker, New York (1970).

Received Apr. 10, 1970. P.S.E.B.M., 1970, Vol. 134.