An effect of isoproterenol on rates of synthesis and secretion of testosterone

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EIK-NES, K. B. An effect of isoproterenol on rates of synthesis and secretion of testosterone. Am. J. Physiol. 217(6): 1764-1770. 1969.—When epinephrine, norepinephrine, ethylnorepinephrine, or isoproterenol was infused at a constant rate via the spermatic artery of the dog, the level of testosterone increased in spermatic venous blood. Of the catecholamines tried, isoproterenol was the strongest stimulator of testosterone secretion, and in infusion experiments of 15 min duration the effect of isoproterenol on testosterone secretion was similar to that following gonadotrophin infusion. Testes infused with isoproterenol via the spermatic artery had higher tissue levels of testosterone than control testes; thus, isoproterenol enhances synthesis de novo of testosterone when infused via the spermatic artery. Since testes infused with a β-receptor inhibitor via the spermatic artery secreted less testosterone than control testes when stimulated with isoproterenol, it is suggested that the integrity of testicular β-receptors may be of importance for the secretion of testosterone in vivo. Currently it is not understood if isoproterenol and the gonadotrophins promote secretion of testosterone by the same mechanism.

during the past 10 years considerable progress has been made in elucidating factors involved in secretion and biosynthesis of gonadal hormones (5). It has been suggested that the gonadotrophins promote increased production of gonadal steroids via the compound cyclic-3',5'-AMP (8, 17) but there are vast lacunae in the concrete knowledge of how this compound acts either on the ovary in vitro or on the testis in vivo. In the mechanistic schemes postulated for gonadotrophic action, a possible intervention of catecholamines has been ignored. Setchell and his co-workers (20) observed, however, that administration of isoproterenol (Isuprel) was associated with increased flow of blood in the testicular artery of the ram; this is a finding of some interest since Eik-Nes (6) noticed that over a considerable range the rate of blood flow in the spermatic artery of the anesthetized, heparinized dog determined the rate of secretion of testosterone by the testis. Spermatocidal arterial blood flow decreases following administration of epinephrine or norepinephrine in the conscious ram (20); and Levin et al. (16) found that when epinephrine was infused in normal men at a rate of 0.466 mg/hr for 3 hr, both the production rate and the plasma level of testosterone decreased significantly. On the other hand, nor-

epinephrine is known to accelerate the rate of secretion of pituitary gonadotrophins in female rats and rabbits (11) and treatment with adrenergic receptor blocking agents will suppress such release (12).

In light of these apparently conflicting data, it was deemed important to study the effects of catecholamines on secretion of testosterone in vivo by the isolated canine testis.

MATERIALS AND METHODS

Experiments were conducted on healthy, mongrel, male dogs ranging in weight between 18 and 24 kg. All animals were anesthetized by intravenous sodium pentobarbital (30 mg/kg). Two animal preparations were used.

Animal preparation 1. The in situ, in vivo preparation of the dog testis has been described in detail elsewhere (6-8). Since substances were to be infused which are known to promote vasorelaxation in the testis (20), the inlet pressure of arterial blood was increased in the spermatic artery.

Animal preparation 2. In order to compare rates of secretion of testosterone by the left and by the right testis of the same dog, the following animal preparation was developed. A cannula was first placed in the right femoral artery of the anesthetized dog and the left and the right spermatic arteries prepared as described in previous communications (6-8). The animal was then heparinized and the cannula in the right femoral artery connected with a constant-rate infusion-withdrawal pump delivering the animal's arterial blood at constant rate to an oxygenator (13). With two additional constant-rate infusion withdrawal pumps, the oxygenated blood was removed from the oxygenator and delivered via individual cannulas into a metabolic chamber maintained at 37.3 C. The spermatic arteries were then cannulated (6-8); the testes were removed from the animal and placed in the metabolic chamber. The cannulas in the spermatic arteries were connected with the cannulas from the constant-rate infusion-withdrawal pumps delivering the animal's arterial blood from the oxygenator. The effluent blood from the infused organs was permitted to drip freely into a container. With a constant-rate infusion pump the substances to be infused via the spermatic arteries were added to the oxygenated, arterial blood shortly before it entered the testicular tissue. This preparation permits the infusion of the right and the left testis of the same dog at controlled rates of arterial blood flow and at controlled temperature. Moreover, the animal's own blood is used for infusion. The testes are, however, without blood circu-
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lation for 30-60 sec during transfer from the dog to the metabolic chamber. As in animal preparation 1 (6, 8) the testes were infused with oxygenated arterial blood for 30 min (30-min presampling infusion) before any experiment was started.

Each testis received oxygenated arterial blood at a constant rate of 3.87 ml/min both in animal preparation 1 and 2. In all experiments close attention was paid to the volume of effluent blood from the infused testes and in no instance did the volume of infused blood differ from that of effluent blood by more than 5%, even in experiments where large amounts of epinephrine or norepinephrine were infused directly into the spermatic artery. Thus, our experimental condition appears to overcome any possible vasoconstrictor effect of the catecholamines on the testicular tissue (20). The indexes R₁ and L₁ in the different figures of this communication indicate the right and the left testis of the same dog.

The animals used in preparations 1 and 2 were given 0.9% sodium chloride solution intravenously at the same rate as that of removal of blood via the femoral artery. The condition of anesthesia was maintained throughout the experiments, with additional sodium pentobarbital injected intravenously over 10 sec.

The blood samples were measured and then centrifuged. The plasma was frozen at once and kept at -15°C until processed for testosterone. Testosterone was determined as described by our laboratory (3, 19). The precision, accuracy, and sensitivity of this testosterone assay have been discussed elsewhere (9).

In a few instances the testicular tissue level of testosterone was determined at the end of infusion. The testes were homogenized in 0.9% sodium chloride solution, the homogenate extracted as previously described (3, 19), and fat was removed from this extract by solvent partition (4). Testosterone was then estimated in the defatted extract by our standard testosterone assay (3, 19).

The following drugs were used: sodium pentobarbital and heparin were purchased from Abbott Laboratories; epinephrine, tyramine, and norepinephrine were purchased from Mann Research Laboratories; phenylethylamine was purchased from Eastman Chemicals; human chorionic gonadotrophin (HCG) and propranolol were gifts from Ayerst Research Laboratories; isoproterenol (Isuprel), ethylnorepinephrine, and 3,4-dihydroxy α-(isopropylamino)-acetophenone were gifts from Winthrop Laboratories; dichloroisoproterenol was a gift from Eli Lilly and Company; N-methylmethaepinephrine was a gift from Dr. M. Armstrong, Fels Research Institute, Yellow Springs, Ohio; and Dibenamine and phenoxybenzamine hydrochloride were gifts from Dr. S. Harvey, Department of Pharmacology, University of Utah, Salt Lake City, Utah.

RESULTS

In Fig. 1 are depicted rates of secretion of testosterone following infusion of 1 μg/min of different compounds via the spermatic artery of the anesthetized dog. Compared to testosterone secretion rates under control conditions (Figs. 2, refs. 4, 7) both epinephrine and norepinephrine appeared to increase testosterone secretion under conditions of controlled input and outflow of spermatic blood. Isoproterenol, however, augmented the secretion of testosterone much faster than either epinephrine or norepinephrine (Figs. 1 and 3). The increase in the secretion of testo-

![Fig. 1. Secretion of testosterone by left testis of 6 different anesthetized dogs following infusion of different compounds via spermatic artery (animal preparation 1). All infusions were made at a constant rate and arterial blood flow to organ was 3.87 ml/min. Temperature of infused organ varied between 37.3 and 37.6°C. During first 30 min of experiment (30-min presampling infusion) 0.38 ml/min of 0.9% sodium chloride solution was added to arterial blood and two spermatic venous blood samples of 1 min duration were collected. Epi — epinephrine, Norepi — norepinephrine, N-methylmetaepi — N-methylmethaepinephrine.](http://ajplegacy.physiology.org/Downloadedfromhttp://ajplegacy.physiology.org/)
FIG. 2. Secretion of testosterone in control animals. Details of this experiment are given in legend of Fig. 1, but at end of 30-min infusion (30-min presampling infusion) saline-blood infusion was continued for an additional 15 min (animal preparation 1).

FIG. 3. Secretion of testosterone by left (L) and by right (R) testis of the same dog. Details of this experiment are given in MATERIALS AND METHODS (animal preparation 2). During first 15 min of experiment each testis was infused with 3.87 ml oxygenated arterial blood and 0.5 ml 0.9% sodium chloride solution/min. At this time isoproterenol (Isuprel), epinephrine, or norepinephrine was added to the arterial blood at a constant rate of 20 &mu;g/min. Compounds were dissolved in 0.9% sodium chloride solution and rate of saline infusion was 0.5 ml/min. Six consecutive samples of spermatic venous blood of 15 min duration were collected from each testis during the 90-min experiment. Epi = epinephrine; Norepi = norepinephrine.

FIG. 4. Percent changes in secretion of testosterone following infusion of different doses of isoproterenol via spermatic artery (animal preparation 1, 9 different dogs). Details of this experiment can be found in legend of Fig. 1, but at end of 30-min presampling infusion period (indicated by arrow) different doses of isoproterenol were infused via spermatic artery at a constant rate for 15 min. Doses of isoproterenol (&mu;g/min) infused are given on the figure. Control = mean percent change in testosterone secretion in 8 dogs infused with 3.87 ml arterial blood and 0.38 ml 0.9% sodium chloride solution/min also during last 15 min of experiment. 1 SEM change in testosterone secretion rates.

testosterone during the first 15 min of isoproterenol infusion (Fig. 4) was rather similar to that observed when small doses of HCG were infused via the spermatic artery for short periods of time (7).

The rates of secretion of testosterone were higher following infusion of ethylnorepinephrine via the spermatic artery than following the same dose of norepinephrine. Moreover, either 3,4-dihydroxy a-(isopropylamino)-aceto-phenone or dichloroisoproterenol promoted secretion of testosterone when administered via the spermatic artery.
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FIG. 5. Secretion of testosterone by left (L) and by right (R) testis of same dog (animal preparation 2). During time 0–90 min one testis (solid line) was infused with 2.1 µg propranolol/min in a mixture of 3.87 ml arterial blood and 0.5 ml saline via spermatic artery. During this period, other testis (dotted line) was infused with a mixture of 3.87 ml arterial blood and 0.5 ml saline/min via spermatic artery. During time 30–90 min both testes were infused with 1 µg isoproterenol (Isuprel)/min via spermatic artery.

Ahlquist (1) postulated that the catecholamines acted on α- or β-adrenergic receptors. This postulate has been amply verified by experiments. Testes infused with the β-receptor blocking agent propranolol (Fig. 5) via the spermatic artery showed less secretion of testosterone when infused with isoproterenol than control testes. With the doses of propranolol and isoproterenol used, the stimulatory effect of isoproterenol on secretion of testosterone could, however, not be completely blocked with propranolol. Testes infused with the α-receptor blocking agent, Dibenamine, via the spermatic artery showed a normal rate of testosterone secretion following infusion with isoproterenol. Of some interest is the observation that testes infused with either Dibenamine or with phenoxybenzamine hydrochloride via the spermatic artery secreted slightly higher amounts of testosterone when infused with epinephrine than control testes (Figs. 6 and 7).

Since isoproterenol is known to increase the flow of blood in the spermatic artery (20), it was important to determine the concentration of testosterone in the testicular tissue at the end of isoproterenol infusions. It is evident from the data of Fig. 8 that such infusions via the spermatic artery resulted in increased concentrations of testosterone in the testicular tissue. This finding strongly suggests that isoproterenol promoted synthesis de novo of testosterone. The rates of secretion of testosterone differed, however, between testes infused with HCG or with isoproterenol via the spermatic artery (Fig. 9). During the first 30 min of infusion, the rates of hormone secretion were quite similar whether isoproterenol or HCG was infused. After this time testosterone secretion by the isoproterenol-infused
testis decreased while that of the HCG-infused organ continued to increase. It is possible that this difference in response is dose dependent; experiments to check this possibility were, however, not performed.

**DISCUSSION**

Under conditions of constant flow of blood in the spermatic artery and inlet pressure in the artery permitting quantitative collection of blood from the spermatic vein, several catecholamines will produce increased secretion of testosterone when administered via the spermatic artery (Figs. 1 and 3). Of the catecholamines tried, isoproterenol was the strongest stimulator of the secretion of testosterone and its effect on hormone secretion over the first 30 min of infusion was quite similar to that of human chorionic gonadotrophin (Fig. 9, ref 7). It was, moreover, demonstrated that testes infused with isoproterenol have higher tissue concentrations of testosterone than control testes (Fig. 8). The conclusion is therefore warranted that isoproterenol will promote both biosynthesis and secretion of testosterone when infused via the spermatic artery. Since the testis in animal preparation 2 (see MATERIALS AND METHODS) is devoid of nerve connection, the effect of isoproterenol on testosterone production cannot depend on intact transmission from the central nervous system. Data have finally been presented supporting the view that the effect of isoproterenol on testosterone secretion probably requires undisturbed β-adrenergic receptors, since testes infused with a β-receptor blocking agent secreted less testosterone following isoproterenol stimulation than control testes (Fig. 5).

Provided that phenoxybenzamine or Dibenamine penetrates the cells producing and secreting testosterone to the same extent as propranolol in our model system in vivo, augmented activity of the α-adrenergic receptors may tend to curb the secretion of testosterone. When α-receptor blocking agents were infused via the spermatic artery, the testosterone secretory response of the testis to epinephrine infusions, but not to isoproterenol infusions, was slightly higher than that of control organs (Figs. 6 and 7). Further experiments must, however, be conducted in order to determine the effect of α-adrenergic receptors on rates of secretion of testosterone. Moreover, we do not know where either the α- or β-receptors are localized in the testis, in Leydig cells, or in Sertoli cells; the former cell type is known to produce and secrete testosterone (14). In previous
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FIG. 9. Secretion of testosterone by left (L) and right (R) testis of same dog (animal preparation 2). During time 15-90 min one testis (solid line) was infused with 20 IU HCG/min while other testis (dotted line) was infused with 1 μg isoproterenol (Isuprel)/min.

work (8) we have reported that when large amounts of the β receptor inhibitor dichloroisoproterenol is infused via the spermatic artery, the response in testosterone secretion to subsequent administration of HCG, but not to cyclic-3',5'-AMP, was partially inhibited. In the current work the administration of a β-receptor inhibitor via the spermatic artery produced diminished response in testosterone secretion following isoproterenol stimulation (Fig. 5). Thus, one may seriously pose the question of significant involvement of adrenergic receptor sites in the male gonad for secretion and probably also for the production of testosterone.

Our model system in vivo is rather specific with regard to catecholamine structure and effect on testosterone secretion. An O-methylated catecholamine is inactive (Fig. 1), whereas the presence of methyl groups on either the α-carbon or on the terminal nitrogen increases the ability of a catecholamine to promote secretion of testosterone in vivo. Dichloroisoproterenol had, as expected (8, 21), no effect on testosterone secretion when administered via the spermatic artery, and the replacement of the β carbon hydroxyl group of isoproterenol with a keto group was associated with complete loss of ability to stimulate androgen secretion. These relationships between structure and function could tend to support the theory of Belleau (2) on a possible role for N-aralkyl-catecholamines in activation of adenylyl cyclase. In accordance with this theory, a catecholamine fixes ATP at the active site and promotes production of cyclic-3',5'-AMP. It is, however, not known if the administration of isoproterenol via the spermatic artery will result in increased production of cyclic 3',5'-AMP in the testicular tissue.

It should, moreover, be remembered that the doses of catecholamines used in the current experiments are rather high, and the observed effects on testosterone secretion may have little to do with the physiological situation in the testis. The concentrations of epinephrine and norepinephrine are low in this organ (10), and nerve fibers associated with the Leydig cells cannot be demonstrated by histological techniques (13). The testicular tissue does, however, contain monoamine oxidase (18) and this enzymic activity is higher in the interstitial cells than in the seminiferous tubules (18).

Since our experiments were done in animals with intact pituitary glands, it may be argued that the observed effect of isoproterenol on testosterone production and secretion could be due to an alteration in the metabolism of the animal’s pituitary gonadotrophin(s). These trophins are delivered to the testis via the arterial blood. Recently we have infused Krebs-Ringer bicarbonate buffer (pH 7.6) via the spermatic arteries in animal preparation 2 (see MATERIALS AND METHODS). If such testes are infused with isoproterenol via the spermatic artery, the level of testosterone in the effluent buffer will increase. No direct measurement has, however, been made of gonadotrophin clearance in the testis in the presence or in the absence of isoproterenol.

Isoproterenol administration via the spermatic artery could promote increased permeability of the testicular cells producing and secreting testosterone. It should, however, be noted that slices of rabbit testis will augment production of testosterone following gonadotropic stimulation without concomitant increase in tissue permeability (5). Currently we have no method for assaying permeability changes in the cells of Leydig. Setchell and associates (20) noted that isoproterenol increased and epinephrine and norepinephrine decreased the flow of blood in the spermatic artery of the ram. No data are, however, available on the effect of these catecholamines on the flow of arterial blood in the microcirculation of the interstitial cells of the testis. If the flow of blood in this circulation is increased following the infusion of catecholamines via the spermatic artery, augmented production and secretion of testosterone would be expected (6, 12). In spite of a great deal of current speculation, it is still far too early to attempt to predict how.
isoproterenol promotes increased production and secretion of testosterone in our model system in vivo.

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