Improved Delivery Through Biological Membranes XIV: Brain-Specific, Sustained Delivery of Testosterone Using a Redox Chemical Delivery System

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Abstract □ The dihydropyridine ⇌ pyridinium salt redox delivery system was used for the specific delivery and sustained release of testosterone in the brain. Administration of the *N*-methyl-1,4-dihydro nicotinate ester of testosterone in female rats gave high and sustained brain levels of the corresponding quaternary ester, testosterone trigonellinate. This contrasted with rapid elimination from the general circulation. Release of testosterone "locked into" brain as the quaternary salt was sustained, $t_{1/2}$ = 20 h.

Keyphrases □ Testosterone—brain-specific delivery, redox chemical delivery system, rats □ Delivery systems—redox chemical, brain-specific sustained delivery of testosterone, rats □ Tissue-specific drug delivery—testosterone in brain tissues, rats, redox chemical delivery system

Our earlier studies with phenylethylamine and dopamine (1–5)[1] established the feasibility of using the biological oxidation of dihydropyridine derivatives to the corresponding quaternary salts as the basis for a potentially selective drug-delivery system. We now report further investigation of this redox delivery concept as it applies to the selective retention and subsequent release of testosterone in the brain. One objective was to extend the redox concept to a larger molecule, testosterone, representative of a biologically important class of compounds, the steroids. A second objective was to study the effects of an ester linkage on the redox properties, distribution, and site-specific delivery of the target drug testosterone. Finally, it is suggested that this type of drug delivery system would also be useful for specific and/or enhanced delivery of drugs to the testes.

BACKGROUND

The selection of testosterone as the target drug and brain as target organ was motivated by our earlier work with phenylethylamine and dopamine derivatives (1–3) and by other considerations, both theoretical and practical. The potentially practical value of selective delivery and sustained release of testosterone in the brain resides in its physiological and biochemical roles in reproductive functions. It has been suggested that testosterone controls male sexual behavior by direct action on the brain (6, 7), and evidence indicates that gonadal steroids are concentrated in brain tissue relative to plasma (8). It has been shown that brains of mammals concentrate sex-related steroids in cells that form a stable core for a hormone-concentrating neural structure (9, 10) and that androgens influence male reproductive behavior by direct action on these discrete areas of the brain known as the anterior hypothalamic–preoptic complex. There is substantial evidence (11, 12) that estradiol is formed from testosterone by aromatization in the preoptic region and that it is the principal, centrally acting steroid in the maintenance of copulatory behavior. The use of androgen to treat breast cancer cases that respond to male hormones is well known (13), but despite the reported antiestrogenic action of testosterone (14), the exact mechanism through which advanced postmenopausal breast cancer responds to testosterone is unknown. A sustained-action form of testosterone that selectively concentrates in brain tissue clearly would be of value in studying the mechanisms through which testosterone exerts its effects on sexual behavior, sexual development, and reproduction. Similarly, it would be of value in studying any possible central role of androgens in the control of breast cancer.

The existence of a blood–testis barrier (BTB) by which some substances are prevented from being carried into the seminiferous tubules long has been suspected (15–18), and some investigators have suggested a similarity between the blood–brain barrier (BBB) and the BTB (19–22). Fawcette *et al.* (23) suggested that the permeability barrier is not in the testis capillary walls because these more closely resemble the capillaries of muscle than those involved in the BBB. Dym and Fawcette (24) concluded that the epithelioid contractile layer around the seminiferous tubules constitutes a significant permeability barrier augmented by an apparently more efficient barrier involving tight cell-to-cell junctions between Sertoli cells that inhibits penetration of substances through the germinal epithelium. Despite such histological differences, pharmacokinetic studies (25, 26) have demonstrated that the functional BTB resembles the BBB in transport characteristics, both depending on lipid solubility and molecular size.

As will be apparent from subsequent considerations, concentration of testosterone in the brain tissues of female rats by biological quaternization is accomplished without significant elevation of serum testosterone levels. The low serum levels would allow one to use such a drug in prepubertal hypoganodism tests, without fear of epiphyseal closure. Similarly, if androgens exert antineoplastic and other effects through central actions, concentration in the brain without elevated peripheral circulatory levels would minimize peripheral side effects such as edema and masculinization.

Our earlier work with phenylethylamine and dopamine derivatives (1–3) led us to conclude that the 17-β(1,4-dihydrotrigonelline) ester of testosterone (I) would, by virtue of its good lipid solubility, cross both the BBB and the BTB (in male rats). As shown in Scheme I, it was also anticipated that biological oxidation to the corresponding quaternary derivative would follow, causing a "lock in" of the corresponding ionic, hydrophilic product in the brain and in the testes in male animals. Conversely, oxidation in locales not involving a permeability barrier would favor rapid clearance from blood because the quaternary derivative (II) is excreted more rapidly than the unoxidized form (I). Thus, oxidation would favor the accumulation of II in the brain while yielding minimal blood levels. A subsequent slow hydrolysis to free testosterone in the brain, therefore, would provide a site-specific, prolonged testosterone action and minimal peripheral effects. In a systematic study of some trigonelline and dihydrotrigonelline esters (III, IV), where R = C_nH_{2n-1} and n = 1–10 (5), we have demonstrated that the quaternary esters (III) hydrolyze in different biological media at rates much slower than those of the uncharged analogues. Esters IV also are very difficult to hydrolyze, and although it was expected that $K_8 \gg K_9$ and $K_5 \gg K_4$, the slow rates of quaternary hydrolysis were expected to ensure a sustained release of testosterone in both testes and brain while yielding minimal levels in blood and other tissues. Earlier studies (1, 2) also led us to expect that trigonelline released in the brain by the hydrolytic process would be excreted easily and at comparable rates (25)[1].

EXPERIMENTAL

All melting points were taken on a melting point apparatus[2] and are uncorrected. Elemental analyses were performed at Atlantic Microlabs, Inc., Atlanta, Georgia. IR spectra were determined using a double-beam recording spectrophotometer[3]. ^{1}H-NMR spectra were determined using a spectrometer[4]. All chemical shifts reported are in δ units (ppm) relative

[1] N. Bodor and M. E. Brewster; unpublished results.

[2] Mel-Temp.
[3] Beckman Acculab 1.
[4] Varian T60A or FX100.

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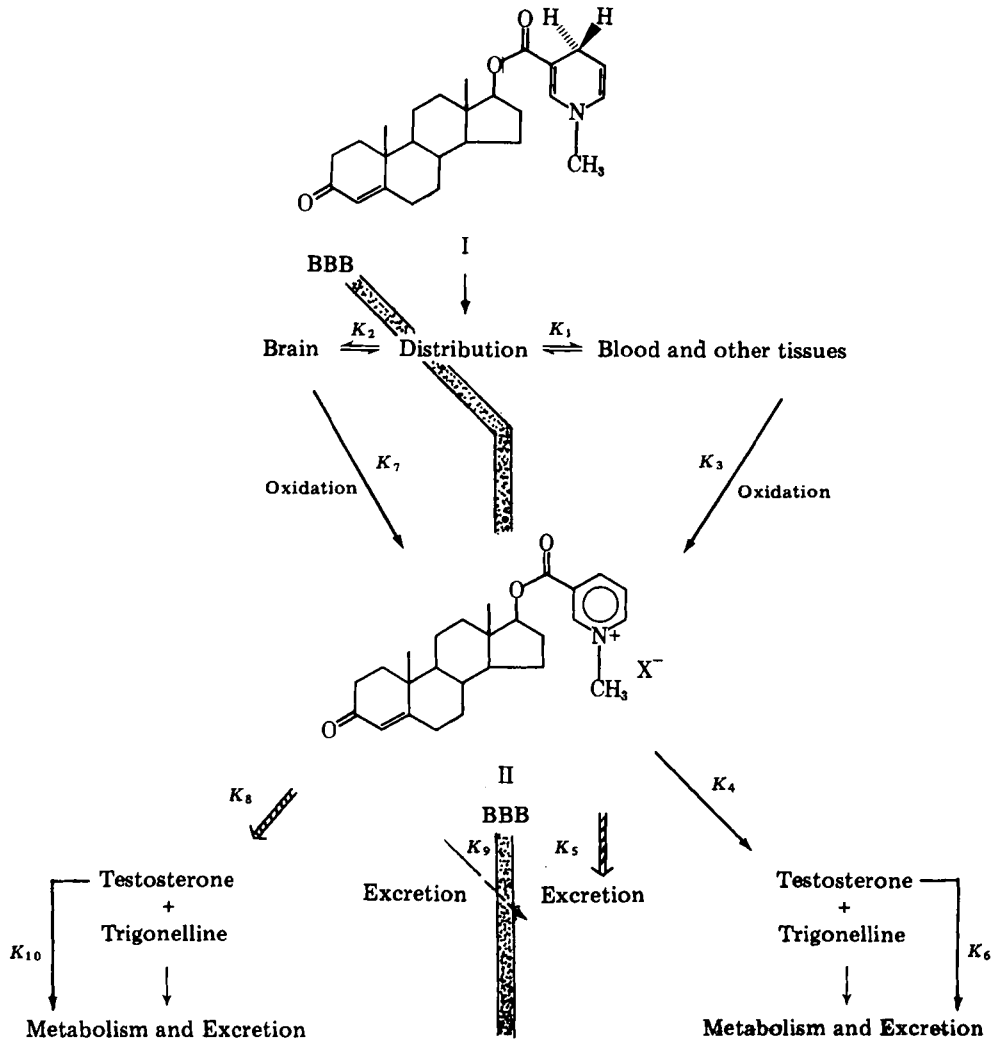
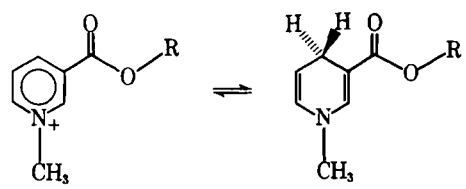


Scheme I

to tetramethylsilane. UV absorbance spectra were determined using a spectrophotometer[5]. HPLC analyses were performed on a ternary liquid chromatograph[6] with a solvent delivery system[7], injector[8], absorbance detector[9], and controller[10].

Testosterone Nicotinate (V)—Thionyl chloride (2.mL) was added to 0.7 g (5.7 mmol) of nicotinic acid[11] and the mixture was refluxed for 3 h. The excess thionyl chloride was removed under reduced pressure, and 10 mL of dry pyridine was then added to the cold residue followed by 1.44 g (5.0 mmol) of testosterone[11]. The mixture was heated with continuous stirring at 100°C for 4 h. The pyridine was removed *in vacuo*, and 5 mL of methanol was added to the oily residue. The mixture was cooled, and the solid that crystallized was filtered and recrystallized from methanol–acetone to give 1.4 g of V as white crystals (yield 71%), mp 187–188°C. This material was used without further purification.

17β-[(1-Methyl-3-pyridiniumcarbonyl) oxy] androst-4-en-3-one Iodide (II)—To a solution of 1.0 g (2.5 mmol) of the aforementioned



III IV

testosterone nicotinate (V) in 15 mL of acetone was added 1 mL of methyl iodide, and the mixture was heated at reflux overnight. The yellow material that separated was removed by filtration, washed with acetone, and crystallized from methanol–ether to yield 1.25 g (92%) of II as yellow crystals, mp 215–220°C (dec.). UV (CH_3OH): λ 270 nm (shoulder), ε = 4579; 240 nm (shoulder), ε = 19,375. ^{1}H-NMR ($CDCl_3$): δ 10.0–8.3 (m, 4, C_5H_5N), 5.73 (s, 1, C_4 vinyl H), 4.86 (s, 3, N—CH_3), and 2.40–1.06 ppm (m, 26, methylene H).

Anal.—Calc. for $C_{26}H_{34}INO_3$: C, 58.32; H, 6.40; N, 2.62. Found: C, 58.17; H, 6.48; N, 2.60.

17β-[(1,4-Dihydro-1-methyl-3-pyridinylcarbonyl)oxy]androst-4-en-3-one (I)—To an ice-cold solution of 1.1 g (2 mmol) of testosterone nicotinate-*N*-methyl iodide (II) in 150 mL of deaerated 10% aqueous methanol were added 0.67 g (8 mmol) of sodium bicarbonate and 1.37 g (8 mmol) of sodium dithionite. The mixture was stirred for 20 min at room temperature, and the separated pale-yellow material was removed by filtration, washed with water, and dried over P_2O_5 under vacuum to yield 0.82 g (98%) of I, mp 172–175°C. UV (CH_3OH): λ 356 nm, ε = 9511. IR (KBr): 1700 and 1660 cm^{-1} (C=O). ^{1}H-NMR (DMSO-d_6): δ 6.90 (br s, 1, C_2 dihydropyridine H), 5.83–5.70 (m, 1, C_6 dihydropyridine H), 5.56 (s, 1, C_4 vinyl H), 4.7–4.33 (m, 1, C_5 dihydropyridine H), 3.26 (br s, 2, C_4 dihydropyridine H), 2.93 (s, 3, N—CH_3), and 2.5–0.83 ppm (m, 26, methylene H with CH_3's at 1.16 and 0.83).

Anal.—Calc. for $C_{26}H_{35}NO_3$: C, 76.25; H, 8.61; N, 3.42. Found: C, 76.07; H, 8.65; N, 3.38.

Analytical Methods—An HPLC method was developed for the studies of the degradation of the quaternary (II) and dihydropyridine derivatives (I) using the system described earlier. The absorbance detector was operated at 254 nm. A 15-cm × 4.6-mm i.d., 5-μm particle size, Ultrasphere reverse-phase C_{18} column[12], operated at ambient tempera-

[5] Model 210; Cary.
[6] Model 345; Beckman.
[7] Model 112; Beckman.
[8] Model 210; Beckman.
[9] Model 160; Beckman.
[10] Model 421; Beckman.
[11] Aldrich Chemical Co., Milwaukee, Wis.
[12] Altex.

Table I—Kinetics of *In Vitro* Oxidation of the Dihydropyridine Ester I to the Quaternary Derivative II in Biological Fluids [a]

Medium	k, s^{-1}	$t_{1/2}$, min	r	Method [b]
80% Plasma	8.12×10^{-5}	142	0.959	A
20% Brain homogenate	1.72×10^{-4}	67	0.997	A
Whole blood	1.74×10^{-4}	66	0.997	A, B

[a] At 37°C, initial concentration of I = 2.4×10^{-4} M. [b] Method A: following appearance of [II]; Method B: following disappearance of [I].

ture, was used for all separations. The mobile phase used for the separation of the dihydropyridine derivative, its degradation products, and oxidation products consisted of a 0.002 M solution of 1-heptanesulfonic acid sodium salt[13] in CH_3CN–0.01 M aqueous Na_2HPO_4 (7:3). At a flow rate of 2.0 mL/min, II has a retention time of 12 min and I has a retention time of 5 min. For the analysis of testosterone in the *in vivo* brain delivery studies, the solvent consisted of a 0.002 M solution of 1-heptanesulfonic acid sodium salt in CH_3CN–0.01 M aqueous Na_2HPO_4 (1:1). At a flow rate of 2.0 mL/min, testosterone has a retention time of 3.3 min and II has a retention time of 36.5 min (very broad peak).

Chemical Oxidation Studies—*By Silver Nitrate*—One milliliter of a 5% methanolic solution of the dihydropyridine derivative (I) was added to 5 mL of saturated methanolic $AgNO_3$ solution. The mixture was shaken, left 10 min for complete oxidation, centrifuged, and the UV spectrum checked.

By Hydrogen Peroxide—To a standardized solution of H_2O_2 (0.18 M) contained in a UV cuvette equilibrated at 37°C was added a solution of dihydropyridine derivative (I) to make a concentration of $\sim 5 \times 10^{-6}$ M. The mixture was thoroughly mixed and monitored for the disappearance of the dihydropyridine maximum at 356 nm using a spectrophotometer[5] interfaced with microprocessor[14] with an enzyme kinetic software package.

By Diphenylpicrylhydrazyl Free Radical—To 2 mL of a 9.3×10^{-5} M solution of 2,2-diphenyl-1-picrylhydrazyl free radical in acetonitrile, equilibrated at 26°C, was added 20 μL of a 1.5×10^{-2} M solution of the dihydropyridine derivative in acetonitrile to make a final concentration of 1.48×10^{-4} M. The mixture was monitored at 515 nm using the same instrumentation as for the hydrogen peroxide method against a reference cuvette containing the same mixture in exactly the same concentrations which was previously prepared and left for at least 10 min (reference for A_∞).

Determination of *In Vitro* Rates of Oxidation of I in Biological Media—*Human Plasma*—The freshly collected plasma used was obtained at the Civitan Regional Blood Center, Inc. (Gainesville, Fla.) and contained ~80% plasma diluted with the anticoagulant citrate phosphate dextrose solution USP. The plasma was stored in a refrigerator and used the next day. One hundred microliters of a freshly prepared 0.024 M solution of I in dimethyl sulfoxide was added to 10 mL of plasma, previously equilibrated to 37°C in a water bath, and mixed thoroughly to result in an initial concentration of 2.4×10^{-4} mol/L. One-milliliter samples of plasma were withdrawn every 20 min from the test medium, added immediately to 5 mL of ice-cold acetonitrile, shaken vigorously, and placed in a freezer. When all samples had been collected, they were centrifuged and the supernatants were filtered through nitrocellulose membrane filters (0.45 μm pore size) and analyzed by HPLC, following the appearance of II (method A).

Human Blood—The freshly collected heparinized blood was obtained at the Civitan Regional Blood Center, Inc. (Gainesville, Fla.). The blood was stored in a refrigerator and used the next day. One hundred microliters of a freshly prepared 0.048 M solution of I in dimethyl sulfoxide was added to 20 mL of blood, previously equilibrated to 37°C in a water bath, and mixed thoroughly to result in an initial concentration of 2.4×10^{-4} mol/L. One-milliliter samples of blood were withdrawn from the test medium every 10 min, added immediately to 5 mL of ice-cold acetonitrile, shaken vigorously, and placed in a freezer. When all samples had been collected, they were centrifuged and the supernatants were filtered using nitrocellulose membrane filters (0.45-μm pore size) and analyzed by HPLC following the appearance of II (method A) and the disappearance of I (method B).

Rat Brain Homogenate—Five female Sprague–Dawley rats were decapitated, and the brains were removed, pooled, weighed (total weight 9.2 g), and homogenized in 36.8 mL of aqueous 0.11 M phosphate buffer, pH 7.4. One hundred microliters of a 0.024 M solution of I in dimethyl sulfoxide was mixed with 20 mL of the homogenate, previously equilibrated to 37°C in a water bath, to result in an initial concentration of 2.4×10^{-4} mol/L. Samples of 1.0 mL were withdrawn every 10 min from the test medium, added immediately to 5 mL of ice-cold acetonitrile, shaken vigorously, and placed in a freezer. When all samples had been collected, they were centrifuged and the supernatants were filtered through nitrocellulose membrane filters (0.45-μm pore size) and analyzed by HPLC (method A).

***In Vitro* Determination of the Site-Specific Conversion of II to Testosterone**—A fresh brain homogenate was prepared as previously described. One hundred microliters of a 0.017 M solution of the quaternary compound (II) in methanol was mixed with 10 mL of the brain homogenate, previously equilibrated to 37°C, to result in an initial concentration of 1.7×10^{-4} M. Samples of 1.0 mL were withdrawn every 20 min from the test medium, added immediately to 5 mL of ice-cold acetonitrile, and placed in a freezer. When all samples had been collected, they were centrifuged, and the supernatant was filtered through a nitrocellulose membrane filter (0.45 μm pore size) and analyzed for the quaternary compound II.

***In Vivo* Brain Delivery of Testosterone Following its Administration**—Female Sprague–Dawley rats with an average weight of 225 ± 10 g were injected intravenously with testosterone at a dose level of 28.2 mg/kg. Samples of brain and blood were collected as previously described (1, 3, 4) and were analyzed for testosterone using HPLC.

Distribution of II after Intravenous Administration—Following the same procedure, female Sprague–Dawley rats were injected intravenously with quaternary solution (0.18%) in dimethyl sulfoxide at a dose level of 13.0 mg/kg. The brain samples collected were analyzed for the presence of quaternary II using HPLC.

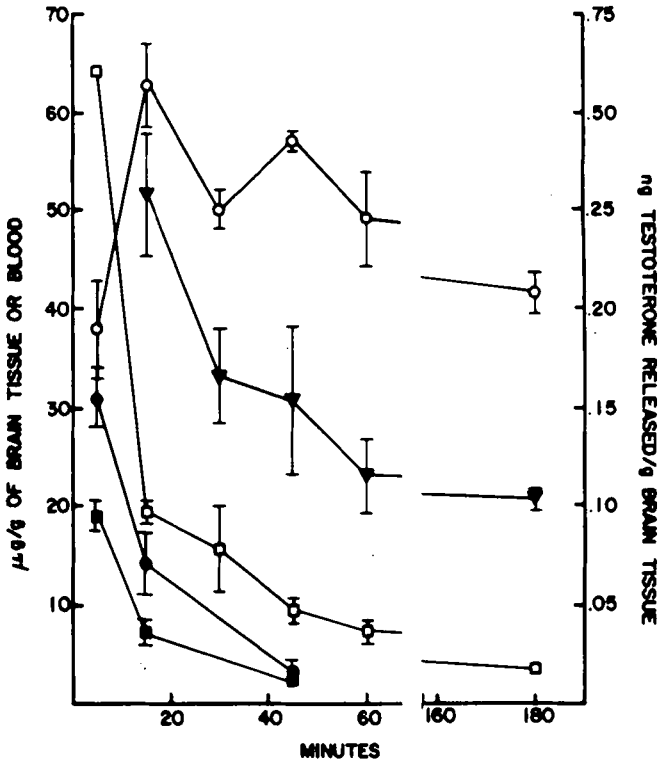


Figure 1—*Concentrations against time for the testosterone-17-nicotinate-N-methyl cation, calculated as iodide, in brain (○) and in blood (□) and concentration of released testosterone (ng/g) in brain (▼) following administration of the corresponding dihydropyridine derivative I. Concentrations of testosterone in brain (●) and blood (■) following administration of testosterone are also included. Bars represent the standard errors.*

RESULTS AND DISCUSSION

Compound I was prepared *via* quaternization of testosterone nicotinate (V) with methyl iodide followed by reduction of the quaternary salt II

[13] PIC B-7; Eastman Kodak Co.
[14] Apple II computer.

using sodium dithionite. The rates of oxidation of the dihydro derivative (I) with silver nitrate, hydrogen peroxide, and diphenylpicrylhydrazyl free radical ($DPP^{\cdot}$) were determined. The reactions were carried out under pseudo-first-order conditions, either with higher concentrations of the oxidant in the case of hydrogen peroxide or higher concentrations of I in the case of the picryl reagent. With $DPP^{\cdot}$ a reference sample was made using the same amounts as the test sample, but prepared 10 min before mixing and monitoring the test sample. This reference is used as a measure of A_∞, and these were the data used to calculate the kinetic parameters. The *in vitro* rates of oxidation of the dihydro derivative were also determined in biological fluids, *e.g.*, 80% plasma, whole blood, 20% brain homogenate, and 20% liver homogenate. The rate of disappearance of the ester II and appearance of testosterone in medium was also determined. Finally, the *in vivo* brain delivery and blood concentration profile of the quaternary derivative and testosterone released, against time, was determined following a single injection of the dihydropyridine derivative (I) to female rats. These results were compared with the blood and brain kinetics of testosterone following administration of the hormone itself.

Chemical Oxidation of the Dihydropyridine Derivative I—*By Silver Nitrate*—It was observed that this dihydro derivative is more stable towards oxidation than the previously studied analogous (1, 3, 4) derivatives containing amido functions; it takes a few minutes standing for the silver to form. The product is exclusively the quaternary salt II, as verified by the change in the UV and NMR spectra.

By Hydrogen Peroxide—Using low concentrations of the dihydro derivative I (5×10^{-6} M) and higher concentrations of the peroxide (0.18 M), the oxidation proceeds according to a first-order kinetics: $k = 2.7 \pm 0.3 \times 10^{-3}\ s^{-1}$; $t_{1/2} = 3.98 \pm 0.7$ min; $r = 0.995$. At higher concentrations I is insoluble in H_2O_2.

By Diphenylpicrylhydrazyl Free Radical—The reaction was carried out under pseudo-first-order conditions using excess dihydropyridine derivative. With the concentrations used, all of the runs gave first-order plots every 3 half-lives, with a correlation coefficient of >0.9998: $k = 4.87 \pm 0.31 \times 10^{-2}$; s^{-1} $t_{1/2} = 14.1 \pm 0.6$ s.

***In Vitro* Oxidation and Hydrolysis in Biological Media**—Table I shows the rates, half-lives, and correlation coefficients for the process of oxidation of the 1,4-dihydropyridine derivative I in different biological media. The rate of hydrolysis of the quaternary II in 20% brain homogenate was also determined, and it was found to be $3.6 \times 10^{-5}\ s^{-1}$, corresponding to a half-life, $t_{1/2}$, of 5.16 h.

***In Vivo* Administration of I and Testosterone**—Figure 1 illustrates the concentrations of quaternary derivative II in brain and blood and the concentration of testosterone released in the brain following intravenous administration of the 1,4-dihydropyridine derivative I. Also, it shows the concentration of testosterone in the brain and blood following administration of testosterone.

17β-[(1,4-Dihydro-1-methyl-3-pyridinylcarbonyl)oxy]androst-4-en-3-one (I) could be obtained in a high yield, >90%, from testosterone 17β-nicotinate by simple chemical procedures. The dihydro product obtained directly from the reduction reaction medium was found to be quite pure by HPLC, and a single crystallization from hot methanol afforded an analytically pure product. No signs of oxidation were observed during crystallization (even from hot methanol), filtration, or drying. The crystalline solid dihydro compound did not show signs of oxidation, decomposition, or polymerization when tested by HPLC during the 2-month shelf storage at ambient temperature under nitrogen. This compound (I) was found to be quantitatively oxidizable to the corresponding quaternary derivative (II), as identified by UV spectrometry, whether by silver nitrate or hydrogen peroxide. The process of oxidation with silver nitrate is slower than that with the dihydropyridine derivatives of phenylethylamine (3) or dopamine (4). Oxidation with hydrogen peroxide or $DPP^{\cdot}$, under pseudo-first-order conditions, was found to take place at measurable rates ($t_{1/2} = 3.98 \pm 0.7$ min and 14.1 ± 0.6 s, respectively) compared with the rates of oxidation of the corresponding phenylethylamine and dopamine derivatives, which were found to be too fast to be monitored using the same procedure. The *in vitro* investigation in biological fluids indicated a facile oxidative conversion of the dihydro form (I) to the corresponding quaternary (II), but at a slower rate than that of the analogous amides of phenylethylamine or dopamine.

For the *in vivo* studies of I, female Sprague–Dawley rats were chosen because the concentration of endogenous testosterone in the blood and brain of these animals is below the range of sensitivity of the procedure used for its analysis. The results shown in Fig. 1 indicate that the dihydro derivative penetrates the BBB and is readily oxidized in the brain to the quaternary precursor II. The *in vivo* rate of oxidation of the dihydro derivative seems faster than that obtained from the *in vitro* experiment. No dihydro derivative could be detected in the brain within the sensitivity limits of the procedure. After II reaches its maximum concentration, within ~15 min, its concentration starts a decline phase corresponding to overall excretion and/or metabolism–hydrolysis. The overall rate of this phase was calculated to be $2 \times 10^{-3}\ min^{-1}$ ($t_{1/2} = 5.7$ h). In the same time the concentration of II in blood was decreasing progressively from the beginning at a rate $1.27 \times 10^{-2}\ min^{-1}$ or with a half-life of 54 min.

Equimolar administration of testosterone using the same solvent (dimethyl sulfoxide) and the same route of administration showed a rapid absorption of testosterone into the brain, reaching a maximum concentration within 5 min, followed by fast clearance from both brain and blood ($t_{1/2}$ = 12.6 and 14.5 min, respectively). The ratio of brain/blood concentration for testosterone was found to be 1.6 at 5 min and 1.8 at 15 min postadministration. The ratio of brain/blood concentration of the quaternary II was found to increase progressively with time (3.23 at 15 min, 6.33 at 45 min, and 12 at 3 h postadministration). This indicates the predicted "lock in" property for the quaternary II.

Testosterone was found to be released from the quaternary ester II and could be detected in the brain following administration of the dihydro derivative I. Analysis of the time concentration curve for release of testosterone (Fig. 1) indicated two-phase kinetics for disappearance from the brain. The first is a fast descending phase at a rate of $1.2 \times 10^{-2}\ min^{-1}$ followed by a slow clearance phase with a rate of $5.8 \times 10^{-4}\ min^{-1}$ and a half-life of ~20 h, which corresponds to ~130 h for complete clearance from the brain. This result, if compared with that obtained by Frey *et al.* (27), for the clearance of testosterone from plasma after oral administration (~6 h), is very promising. Although the concentrations of testosterone in the brain following administration of I are low compared with that following administration of testosterone, this is by no means a disadvantage, because such high concentration of testosterone may not be needed for receptor saturation. By dose manipulation of the dihydro derivative, a concentration of testosterone just sufficient for receptor saturation for a delayed period could be attained.

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Angular Dependence of the Interaction Energy Between the N Lone Pair of Amines and a Proton: Relevance to Drug–Receptor Systems

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Abstract □ *Ab initio* molecular orbital calculations with a 4-31G basis set have been performed to study the angular dependence of the interaction energy between a lone electron pair of nitrogen and a proton. In this study ammonia and trimethylamine were used as models of biologically active amines. A proton was used as a model of an electrophilic site at the receptor. Results obtained confirm previous indications that the energy required to bend the proton from the lone pair direction decreases markedly as the two species are further separated from one another. Implications regarding the interactions of drugs and hormones at specific receptors are discussed.

Keyphrases □ Interaction energy—nitrogen lone electron pair of amines and a proton, angular dependency, drug–receptor systems □ Drug–receptor systems—interaction energy of the nitrogen lone electron pair of amines and a proton, angular dependency □ Amines—biologically active, interaction energy of the lone electron pair and a proton, angular dependency

Many hormones and drugs elicit their biological response through interactions with specific receptors (1–10). These interactions are typically weak, reversible, and specific (11) and, in addition, do not involve covalent bonding (1, 2, 8, 9). Many such interactions are of the electrophile–nucleophile type between sterically fixed groups. Because the nucleophilic pharmacophore frequently contains nitrogen at a critical position, we focus here specifically on the nature of the interaction of nitrogen with an electrophile.

One way nitrogen could interact with the specific electrophilic center at the receptor is *via* its lone electron pair. Such a mechanism has been suggested for opiate–receptor interactions (12–15). The influence of the directionality of the lone pair of biologically active amines on their activity has been demonstrated experimentally in some cases (12). One may envisage the most productive interaction as that in which the N lone pair is aligned exactly in the direction of the electrophilic site. This concept of amine–receptor interaction is illustrated in Fig. 1, which schematically depicts the binding of a (tertiary) amine to its receptor. Both the amine molecule and the electrophilic site are visualized as being sterically fixed at the receptor. We consider the distance between the N lone pair and the electrophilic site as longer than the normal bonding distance, since covalent bonding of nitrogen to the receptor does not occur (1). Figure 1A shows a perfect fit between the N lone pair and the electrophilic site. Figure 1B depicts a case of a substituted amine whose substituent (shown as a "bump" at the left-hand side of the molecule) interferes with the proper fit with the receptor cavity. There may of course be other factors leading to poor fit, and the effect shown in Fig. 1B is used only as an example. The repulsion between the substituent and the receptor cavity causes a small tilting of the molecule, which changes the position of the nitrogen atom and, therefore, its lone electron pair, relative to the electrophilic site. The N lone pair forms a "bent" complex with the electrophilic site.

Since the activity of the amine depends on the nucleophile–electrophile complexation, it is very important to learn about the energetics of the "bent" complexation of

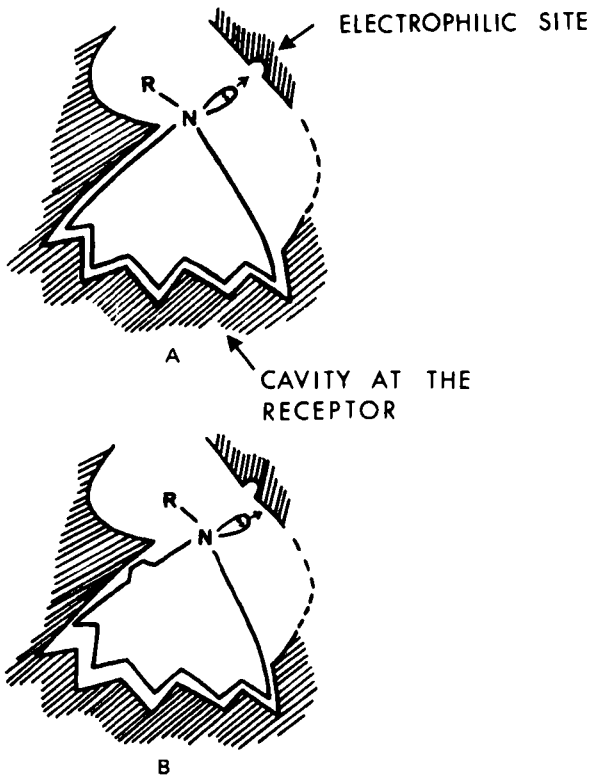

Figure 1—*Schematic representation of the binding of a biologically active (tertiary) amine to its receptor. Key: (A) perfect fit between the amine and its receptor; (B) less than perfect fit due to presence of a substituent (shown as bump on left of molecule).*

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