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SHORT COMMUNICATIONS

Extraction of AZT and dideoxynucleosides by rat prostate but not testis

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Azidothymidine (3′-azido-3′-deoxythymidine; AZT or zidovudine [Retrovir]) is a nucleoside analogue that inhibits the replication of the human immunodeficiency virus (HIV), the etiologic agent of the acquired immunodeficiency syndrome (AIDS) [1]. Likewise, the dideoxynucleosides 2′,3′-dideoxycytidine (DDC) and 2′,3′-dideoxyadenosine (DDA) are being studied as antiviral agents in the treatment of AIDS [2–4]. HIV has been isolated from semen of infected males and is the proposed vehicle of sexual transmission among males, or from male to female partners [5–7]. AZT has been found in semen of treated AIDS patients [8] and is presumed to penetrate into prostate and testis, the major organs from which seminal plasma is derived. The ability of AZT, DDC, or DDA to cross the prostate cell membrane or seminiferous tubule may be dependent on organ specific permeability properties. The prostate epithelial cell membrane and the microvascular surface are potential sites of restrictive influx of compounds into prostate. The testis has a unique selective partition, the blood–testis barrier [9], which is formed by epithelial-like tight junctions between adjacent Sertoli cells. The blood–testis barrier thus segregates the seminiferous tubule from the surrounding microvascular and interstitial space [10].

The present studies were undertaken to examine prostate cell and seminferous tubule extraction of AZT, DDC, and DDA in the rat, utilizing an *in vivo*, single injection tissue sampling technique.

Materials and Methods

Materials. The radiolabeled drugs were obtained from Moravek Biochemicals Inc. (Brea, CA). The manufacturer specific activities were: [^{3}H]AZT, 10 Ci/mmol; [^{3}H]DDC, 5 Ci/mmol; and [^{3}H]DDA, 30 Ci/mmol. [^{3}H]$NaBH_4$, 11.5 Ci/mmol, and *n*-[1-^{14}C]butanol, 0.88 Ci/mmol, were obtained from the New England Nuclear Corp. (Boston, MA). The radiochemical purity of the isotopes was 99% as assessed by thin-layer chromatography with radiochromatogram scanning (Packard model 7230 radiochromatogram scanner, Packard Instrument Co., Downers Grove, IL). Bovine albumin was tritiated by reductive methylation using [^{3}H]$NaBH_4$ to a specific activity of 0.3 μCi/μg [11].

Arterial injection technique. A tissue sampling, single injection technique was utilized to quantitate tissue uptake [12]. Abdominal aortic injections, just proximal to the origin of the spermatic artery, were made in adult male Sprague–Dawley rats (175–275 g body wt) under ketamine/xylazine anesthesia (ketamine 235 mg/kg, i.m.; xylazine 2.3 mg/kg, i.m.). Approximately, 200 μl volumes were rapidly injected as a bolus into the aorta via a 27-gauge needle. The injection solution contained 12–16 μCi/ml of the test isotopes ([^{3}H]AZT, [^{3}H]DDC, [^{3}H]DDA, or 5–10 μCi/ml of [^{3}H]albumin) along with 1–2 μCi/ml of [^{14}C]butanol, a highly diffusible reference compound. The radioisotopes were mixed in an injection solution of 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)-buffered Ringer's solution, pH 7.4, containing 0.1% bovine albumin.

At 15 sec after injection, the animals were killed by transection of the aorta, and the testis and prostate were removed quickly. Testicular or prostate tissue was solubilized in 2.0 ml soluene-350 (Packard Instrument Co.) at 50° for 90 min. An aliquot of the injection solution was similarly treated, and testis or prostate and the injection solution were analyzed by double isotope (^{3}H and ^{14}C) liquid scintillation counting. The percent prostate uptake index (PUI), or testis uptake index (TUI), was calculated:

$$\text{PUI or TUI} = \frac{^3\text{H}/^{14}\text{C dpm prostate or testis}}{^3\text{H}/^{14}\text{C dpm injectate}} \times 100.$$

The PUI or TUI = E_T/E_R, where E_T and E_R represent the organ extraction of the test compound and the reference compound respectively. The E_T values were computed from the E_T = PUI or TUI × (E_R) product, where E_R values for butanol are 82 ± 5 and 81 ± 2% for the prostate and testis respectively [12]. For both prostate and testis the extraction of butanol reached maximal values at 15 sec [12], indicating complete passage of the injection bolus through the microvasculature.

For the prostate, the percent cellular extraction (E) of a test compound by the prostate can be calculated if the extractions of vascular and interstitial markers is known. Albumin is an example of a compound that can traverse prostatic capillaries and enter the interstitial space, but is excluded from the prostate epithelial cell [13]. Therefore, the percent extraction (E), due to prostate cell uptake of the drug, was computed as follows:

$$E = \frac{E_T\text{hormone} - E_T\text{albumin}}{100 - E_T\text{albumin}} \times 100.$$

Data presented are mean ± SEM. Statistical significance was assessed by analysis of variance.

Results and discussion

For the prostate, the PUI values were: [^{3}H]AZT = 90.8 ± 7.7, [^{3}H]DDC = 109 ± 4.3, [^{3}H]DDA = 91.2 ± 2.9, and [^{3}H]albumin = 48.0 ± 2.8%. The E_T values for these compounds are shown in Fig. 1. The extractions of AZT, DDC, and DDA were significantly higher than that for albumin ($P < 0.01$). DDC was found to have the highest prostatic extraction of the three nucleosides ($P < 0.05$). The prostatic cellular extractions (E) for AZT, DDC, and DDA were 58 ± 10.4, 82.6 ± 5.8, and 58.4 ± 3.9% respectively (Fig. 2). The E value for DDC was significantly greater than for AZT or DDA ($P < 0.05$).

For the testis, the TUI values were: [^{3}H]AZT = 18.3 ± 1.9, [^{3}H]DDC = 26.4 ± 3.8, [^{3}H]DDA = 26.3 ± 4.8, and [^{3}H]albumin = 43.3 ± 1.7%. The E_T values for these compounds are shown in Fig. 1. The extractions of AZT, DDC, and DDA were not significantly different from each other, but were significantly lower than albumin ($P < 0.05$).

The present studies indicate that AZT, DDC, and DDA are readily extracted by the rat prostate cell, but not by the seminiferous tubule, i.e. across the blood–tubular barrier. Prostatic capillaries are permeable to large plasma proteins, such as albumin and transferrin [12], which necessitates conversion of E_T values, which represent total organ extraction, to E values which represent prostate cell extraction, i.e. unidirectional clearance across the prostate epithelial

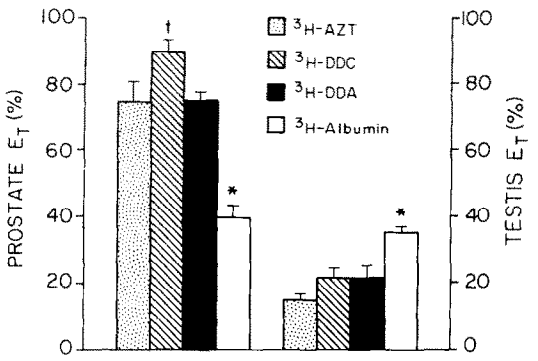

Fig. 1. Percent prostatic or testicular extraction (E_T) of [^{3}H]AZT, [^{3}H]DDC, [^{3}H]DDA, and [^{3}H]albumin. E_T = (PUI or TUI) × E_R, where E_R for the butanol reference is 0.82 (prostate) or 0.81 (testis). Data are means ± SEM (N = 4–7 rats); Fisher's LSD comparison: * $P < 0.01$ and † $P < 0.05$.

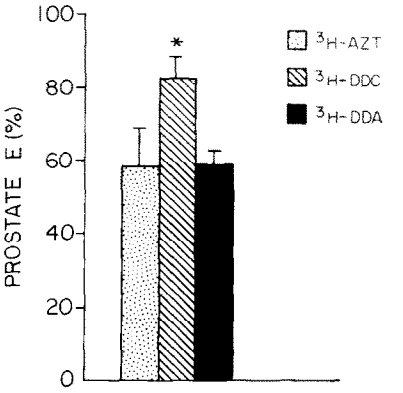

Fig. 2. Percent prostatic cellular extraction (E) of [^{3}H]AZT, [^{3}H]DDC, and [^{3}H]DDA. Data are means ± SEM (N = 5–6 rats). Key: (*)$P < 0.05$.

cell membrane (see Materials and Methods). AZT, DDC, and DDA showed marked extraction (E) by the prostate cell (Fig. 2). The microvascular permeability of the prostate is similar to the liver capillary wall [14]. Terasaki and Pardridge [15] have reported first pass hepatic extraction in the rat for AZT, DDC, and DDA to be 88, 26 and 88% respectively.

Neither AZT, DDC, nor DDA was extracted by the rat seminferous tubule, i.e. across the blood–tubular barrier. All nucleosides tested were found to have testicular extractions (E_T) less than albumin, and, therefore, AZT, DDC, and DDA appear confined to the microvascular and possibly a portion of the interstitial space. The exclusion of AZT, DDC, and DDA by the blood–tubular barrier was similar to that by the rat blood–brain barrier [15]. The differential extraction of AZT, DDC, and DDA by the prostate but not seminiferous tubule may be important in organ specific efficacy of these agents. Additionally, the prostate cell may be susceptible to potential adverse effects of these nucleoside analogs, whereas the selective impermeability of the blood–tubular barrier may be protective for Sertoli cells and developing spermatozoa.

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REFERENCES

1. Mitsuya H, Weinhold KJ, Furman PA, St. Clair MH, Nusinoff-Lehrman S, Gallo RC, Bolognesi D, Barry DW and Broder S, 3′-Azido-3′-deoxythymidine (BW A509U): An antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotrophic virus type III/lymphadenopathy-associated virus *in vitro*. *Proc Natl Acad Sci USA* **82**: 7096–7100, 1985.
2. Mitsuya H and Broder S, Inhibition of the *in vitro* infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by 2′,3′-dideoxynucleosides. *Proc Natl Acad Sci USA* **83**: 1911–1915, 1986.
3. Starnes MC and Cheng YC, Cellular metabolism of 2′,3′-dideoxycytidine, a compound active against human immunodeficiency virus *in vitro*. *J Biol Chem* **262**: 988–991, 1987.
4. Lin T-S, Schinazi RF, Chen MS, Kinney-Thomas E and Prusoff WH, Antiviral activity of 2′,3′-dideoxycytidin-2′-ene (2′,3′-dideoxy-2′,3′-didehydrocytidine) against human immunodeficiency virus *in vitro*. *Biochem Pharmacol* **36**: 311–316, 1987.
5. Zagury D, Bernard J, Leibowitch J, Safai B, Groopman JE, Feldman M, Sarngadharan MG and Gallo RC, HTLV-III in cells cultured from semen of two patients with AIDS. *Science* **226**: 449–451, 1984.
6. Ho DD, Schooley RT, Rota TR, Kaplan JC and Flynn T, HTLV-III in the semen and blood of a healthy homosexual man. *Science* **226**: 451–453, 1984.
7. Stewart GJ, Cunningham AL, Driscoll GL, Tyler JPP, Barr JA, Gold J and Lamont BJ, Transmission of human T-cell lymphotropic virus type III (HTLV-III) by artificial insemination by donor. *Lancet* **2**: 581–584, 1985.
8. Henry K, Chinnock BJ, Quinn RP, Fletcher CV, de Miranda P and Balfour HH, Concurrent zidovudine levels in semen and serum determined by radioimmunoassay in patients with AIDS or AIDS-related complex. *JAMA* **259**: 3023–3026, 1988.
9. Setchell BP and Waites GMH, The blood–testis barrier. In: *Endocrinology-Male Reproductive System. Handbook of Physiology* (Eds. Greep RO and Astwood EB), pp. 143–172. American Physiological Society, Washington, DC, 1975.
10. Dym M and Fawcett DW, The blood–testis barrier in the rat and the physiological compartmentation of the seminiferous epithelium. *Biol Reprod* **3**: 308–326, 1970.
11. Pardridge WM, Eisenberg J, Fierer G and Musto NA, Developmental changes in brain and serum binding of testosterone and in brain capillary uptake of testosterone binding serum proteins in the rabbit. *Dev Brain Res* **38**: 245–253, 1988.
12. Sakiyama R, Pardridge WM and Musto NA, Influx of

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TeBG and TeBG-bound sex steroid hormones into rat testis and prostate. *J Clin Endocrinol Metab* **67**: 98–103, 1988.
13. Wilson EM, French FS and Petrusz P, Transferrin in the rat prostate Dunning tumor. *Cancer Res* **42**: 243–251, 1982.
14. Goresky CA, The processes of cellular uptake and exchange in the liver. *Fed Proc* **41**: 3033–3039, 1982.
15. Terasaki T and Pardridge WM, Restricted transport of 3′-azido-3′-deoxythymidine and dideoxynucleosides through the blood–brain barrier. *J Infect Dis* **158**: 630–632, 1988.

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Inhibition of dopamine β-hydroxylase by captopril

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The exact mechanism of action of captopril is still unclear. The original supposition that its antihypertensive effect is the result of the sole inhibition of angiotensin converting enzyme has been questioned by several authors (see Refs. 1–3 for reviews). Convincing evidence has accumulated that captopril acts also through a reduction of the sympathetic tone, an effect in part unrelated to converting enzyme inhibition [3]. For example, Antoniaccio and coworkers showed that captopril, but not saralasin or other converting enzyme inhibitors, such as tetprotide and enalapril, causes prejunctional inhibition of noradrenaline release [4] and inhibition of the pressor responses to sympathetic nerve stimulation [5] in pithed spontaneously hypertensive rats. These results were confirmed *in vitro* by Collis and Keddie [6] and Clough *et al.* [7] who, using the model of isolated rat mesenteric arteries, showed that captopril, at concentrations of 100–300 μM, markedly attenuates, by an angiotensin-independent mechanism, the vasoconstriction induced by sympathetic nerve stimulation. It was suggested that the sulfhydryl moiety of captopril is responsible for such effects, since enalapril, that contains no sulfhydryl groups, was found devoid of any effect on sympathetic function [5]. Sulfhydryl compounds have long been known to inhibit, both *in vitro* and *in vivo*, dopamine β-hydroxylase (DBH; 3,4-dihydroxy-phenylethylamine, ascorbate: oxygen oxidoreductase (β-hydroxylating), EC 1.14.17.1) the enzyme that converts dopamine to noradrenaline [8–10]. Inhibition of DBH has been shown to result in decreased sympathetic activity and marked hypotensive effects (see Ref. 10 and references therein). We, therefore, tested the effect of captopril on this enzyme and investigated its mechanism of inhibition. Enalalprilic acid, the pharmacologically active species of enalapril [1], was also tested in order to assess the relevance of the sulfhydryl group to the observed effects.

Materials and methods

Bovine adrenal dopamine β-hydroxylase and all components of the reaction mixtures were obtained from Sigma. Enzyme activity was determined with tyramine as substrate by a modification [9] of the spectrophotometric method of Pisano *et al.* [11]. The kinetic properties of the enzyme preparation used proved to be quite similar to those of the other currently used preparations (pH optimum 5.2; K_m values for tyramine and ascorbate 1.4 and 0.9 mM, respectively). Except when otherwise specified, reaction mixtures contained, in a final volume of 0.5 ml, 100 mM phosphate buffer pH 5.2, 3 mM ascorbate, 4 mM tyramine and sufficient catalase (1000–1500 units) to give maximal stimulation of enzyme activity. After thermal equilibration of the incubation medium at 37°, the reaction was started by the addition of 1–1.5 μg of enzyme and stopped after 10 min by the addition of 0.1 ml of 50% trichloroacetic acid. It was verified that product formation was linear with time also in conditions of maximum substrate depletion, which was never allowed to exceed 5%. Initial enzyme velocity at saturating substrate concentrations was about 3 μmol per min per mg of protein. Protein concentration was determined according to Lowry *et al.* [12]. All data presented are the mean of three to four experiments performed in duplicate. The interassay error was less than 8% coefficient of variation. Regression lines were determined by a weighed least square method.

Results and discussion

From a dose–response curve, the captopril concentration giving 50% inhibition of DBH activity (IC_{50}) was determined to be 140 μM. Enalaprilic acid had no effect on the enzyme activity up to 2 mM. The inhibitory effect of captopril was found fully reversible upon dilution. In addition, the inhibition was found to be independent of time when the time of incubation was varied from 2 to 20 min. These results are indicative of a truly reversible interaction between enzyme and inhibitor. The effect of captopril on DBH kinetics is shown in Fig. 1 in the form of a reciprocal velocity plot vs the reciprocal of the concentration of ascorbate. This plot indicates noncompetitive inhibition with respect to ascorbate, according to the definition of Cleland [13]. The double-reciprocal plot of Fig. 2 shows that captopril behaved as a noncompetitive inhibitor also

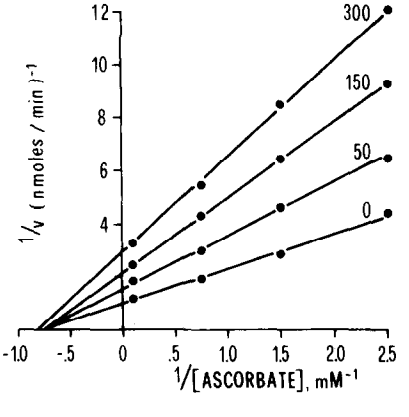

Fig. 1. Plots of reciprocal of initial velocity vs reciprocal of ascorbate concentration in the absence and presence of captopril. Ascorbate concentration was varied from 0.4 to 10 mM, whereas tyramine concentration was held constant at 4 mM. The numbers above the lines indicate captopril concentrations in micromolar units.