Synthesis of 4,4-Ditritio-(+)-nicotine: Comparative Binding and Distribution Studies with Natural Enantiomer

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Abstract □ The preparation of 4,4-ditritio-(+)-nicotine (V*b*) (specific activity 10.3 Ci/mmole) from (+)-nicotine (I*b*) *via* (−)-4,4-dibromocotinine (III*b*) is described. Although I*b* is 10–30 times less potent than (−)-nicotine (I*a*) in the CNS, its binding affinity for the crude mitochondrial or nuclear fraction of whole rat brain is only three times less than that of I*a*. However, distribution studies showed that the maximum brain levels of (−)-[^{3}H]nicotine are nearly twice those of (+)-[^{3}H]-nicotine following administration of a 2-μg/kg dose. Binding affinity and disposition of the stereoisomers account for a portion of the pharmacological stereospecificity of nicotine.

Keyphrases □ 4,4-Ditritio-(+)-nicotine — synthesis, comparative binding and distribution with the natural enantiomer □ Stereoisomers—nicotine, binding affinity and disposition in rats □ Binding—nicotine stereoisomers, rats

The actions of nicotine on the central nervous system have been shown to be stereospecific (1–4). If nicotine's effects result from its interaction with receptors, then nicotine binding to brain tissue should reflect this selectivity. Recently (5), a synthesis and some preliminary binding data for 4,4-ditritio-(−)-nicotine (V*a*, specific activity 4.7 Ci/mmole) were presented. In those binding studies, excess (+)- and (−)-nicotine were equally effective in displacing (−)-[^{3}H]nicotine that was bound to rat brain, a finding that suggested nicotine binding lacked stereospecificity.

The stereospecificity of nicotine can be investigated further by comparing directly the binding and disposition of (+)- and (−)-[^{3}H]nicotine. The synthesis of radiolabeled (+)-nicotine, the enantiomer of V*a*, 4,4-ditritio-(−)-nicotine[1] (V*b*), prepared from (+)-nicotine according to Scheme I is reported here. An improved procedure for the hydrogenolysis of (+)-4,4- dibromocotinine (III*a*)[2], which was applied to the tritiolysis of III*b*, the enantiomer of III*a*, is also included. The resultant V*b* with a relatively high specific activity (10.3 Ci/mmole) has facilitated binding and disposition experiments with (−)- and (+)-nicotine (I*a* and I*b*, respectively).

EXPERIMENTAL

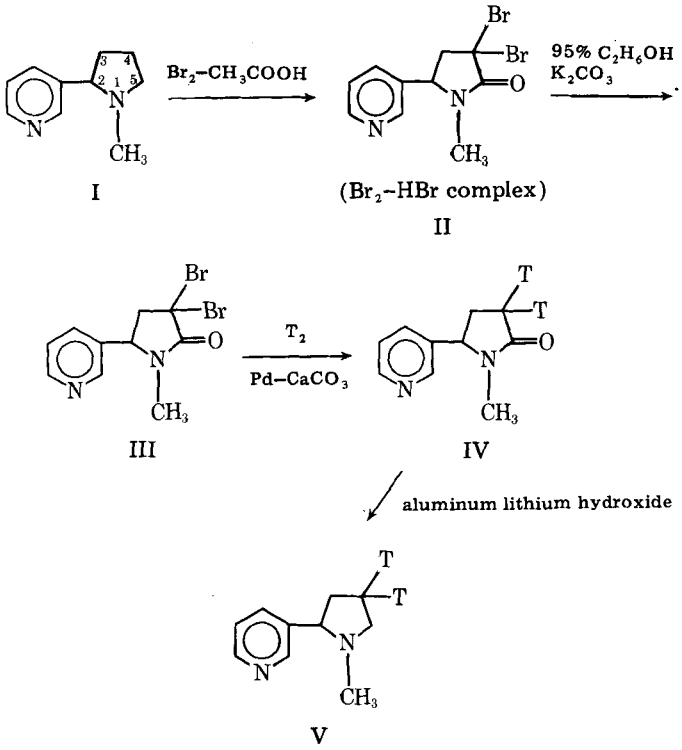
Chemistry—*Conversion of (+)-4,4-Dibromocotinine (III*a*) to (−)-Nicotine (I*a*)*—Compound III*a*[2] (35 mg, 0.2 mmole), $[\alpha]_D^{25}$ +33.44°, $[\alpha]_{546}^{25}$ nm +39.48° (*c*, 2.234 in methanol)[3], prepared from I*a* (5, 6), in 0.5 ml of tetrahydrofuran was added to a suspension of 30 mg of 10% palladium-on charcoal, 0.03 ml (0.2 mmole) of triethylamine, and 1.0 ml of tetrahydrofuran under hydrogen. During 45–50 min of agitation, 4.4–4.6 ml (theory, 4.4 ml) of hydrogen was consumed. Filtration and evaporation of solvent *in vacuo* (without heating) gave (−)-cotinine (6) (essentially pure by TLC)[4], which was reduced to I*a* with aluminum lithium hydride (5).

4,4-Ditritio-(+)-nicotine (V*b*)—Conversion of I*b* (2) to (−)-4,4-dibromocotinine (III*b*), $[\alpha]_D^{25}$ −32.74, $[\alpha]_{546}^{25}$ nm −40.12 (*c*, 0.678 in methanol) (see corresponding values for III*a*), mp 121–122°, was effected in a 50% yield as described previously for the (−)-series (5), I*a* → III*a*. The resulting III*b* was tritiolyzed[5] as described for the hydrogenolysis of III*a*. This tritiated material, IV*b*, was reduced to V*b* with aluminum lithium hydride as described for IV*a* → V*a* (5). The purity of V*b* was determined by TLC[4]. Ninety-nine percent of the radioactivity corresponded to authentic (+)-nicotine (2) and none to cotinine.

*Determination of Specific Activity of V*b—As described previously for V*a* (5), V*b* was injected into a gas chromatograph–mass spectrometer[6] for single-ion monitoring of *m/z* 84, 86, and 88, the base peaks of [$^3H_0$]-V*b*, [$^3H_1$]-V*b*, and [$^3H_2$]-V*b*, respectively. From a [$^3H_0$]-V*b* calibration curve, it was determined that each microcurie of radiolabeled material contained 111, 41, and 5 ng of [$^3H_0$]-V*b*, [$^3H_1$]-V*b*, and [$^3H_2$]-V*b*, respectively. This translates into a specific activity of 10.3 Ci/mmole for V*b*.

Binding Studies—Brains from Sprague–Dawley rats were homogenized in isotonic sucrose, and crude nuclear pellets were obtained by centrifuging the homogenates at 1000×*g*. The supernates were spun at 10,000×*g* to provide crude mitochondrial pellets. The crude nuclear and crude mitochondrial pellets were resuspended in Krebs–Henseleit buffer (pH 8.4) to a final protein concentration of 3 mg/ml. The resuspended fractions were pipetted into microfuge tubes; then saline, unlabeled (+)-nicotine or unlabeled (−)-nicotine was added for a 5-min incubation period at 2°. Then radiolabeled nicotine was added for an additional 5-min incubation at 2°.

Bound and free nicotine were separated by centrifugation in a micro-



Scheme I—a (−) and b (+) series

[1] (+)-Nicotine has the *R* configuration.

[2] Erroneously recorded as (−)-4,4-dibromocotinine in Ref. 5.

[3] E. R. Bowman, Department of Pharmacology, Medical College of Virginia (personal communication) found $[\alpha]_{546}^{25}$ nm + 35.68° (*c*, 12.5 in ethanol) for III*a*.

[4] Silica gel plates GHLF from Analtech (Newark, Delaware) were developed in acetone–ether–concentrated ammonia (4:16:0.1). Nicotine R_f was 0.55.

[5] Amersham-Searle, Arlington Heights, Ill.

[6] Finnigan 4000, Finnigan, Sunnyvale, Calif.

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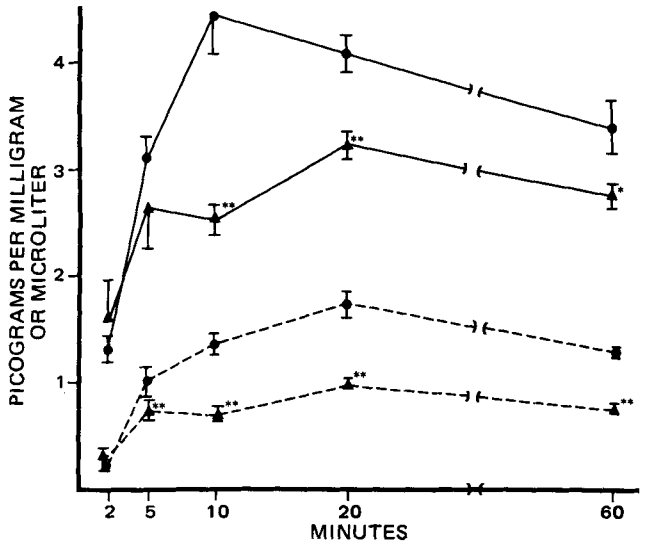

Figure 1—*Time course of (+)- and (−)-[^{3}H]nicotine (2 μg/kg) in rat brain and plasma. Results are expressed as mean ±SE (n = 5). Values are significantly different at p <0.005 (*) and p <0.001 (**) according to a Student t test. Key: (+)-[^{3}H]Nicotine, in brain (▲----▲) and plasma (▲—▲), and (−)-[^{3}H]nicotine, in brain (■----■) and plasma (●—●).*

fuge[7] for 3 min. The tips of the centrifuge tubes, which contained the pellets, were severed with a guillotine and vortexed with scintillation fluid (two parts of toluene containing 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene and one part of alkylphenoxypolyethoxyethanol[8]). Radioactivity was quantitated by liquid scintillation spectrometry, and quench was corrected by external standardization.

Distribution Studies—Sprague–Dawley rats were injected subcutanously with either (+)- or (−)-[^{3}H]nicotine ditartrate in saline (60 μCi/2 μg/kg, calculated as free base), and five rats were decapitated at each time point for each treatment. Blood from the cervical wound was collected in heparinized tubes, which was centrifuged at 1000×*g* to obtain plasma. Brains were homogenized[9] in five volumes of 0.5 *N* HCl. Total radioactivity was determined by counting 100 μl of plasma and tissue homogenates in aqueous counting scintillant[5]. An extraction technique that removed nicotine, but not metabolites, was used to quantitate [^{3}H]nicotine (7). Plasma (0.5 ml) and tissue homogenates (1.0 ml) were made basic with 3 drops of concentrated ammonia and 1 ml of 40% tribasic potassium phosphate. Nicotine ditartrate (1 mg/200 μl) was added as a carrier, and the samples were shaken with 10 ml of hexane. Hexane (8 ml) was removed and counted directly in 10 ml of scintillation fluid. Quench was corrected using external standardization. This extraction removed 90–95% of [^{3}H]nicotine, and no metabolites were detected in these extracts by TLC analysis[4].

RESULTS AND DISCUSSION

Binding Studies—The high specific activity of (+)-[^{3}H]nicotine provided the opportunity to compare the binding characteristics and affinity of (+)-[^{3}H]nicotine to those of (−)-[^{3}H]nicotine. The physiochemical requirements for (+)-[^{3}H]nicotine binding to the crude nuclear and crude mitochondrial fractions of whole rat brain were similar to those described previously for (−)-[^{3}H]nicotine (5). Excess concentrations (10^{-4} *M*) of the unlabeled stereoisomers were equally effective in displacing (+)-[^{3}H]nicotine. The quantity of saturable binding of (+)-[^{3}H]nicotine was less than that of (−)-[^{3}H]nicotine, while the amount of nonsaturable binding was similar for both. The ratio of saturable to nonsaturable binding was 1.06 and 0.64 for (−)-[^{3}H]nicotine and (+)-[^{3}H]nicotine, respectively. Scatchard plots of the saturable binding of (+)-[^{3}H]nicotine to the crude mitochondrial fraction revealed a K_D value of 2.2×10^{-7} *M*, which was approximately three times greater than that of (−)-[^{3}H]nicotine ($K_D = 6.3 \times 10^{-8}$ *M*). Binding to the crude nuclear fraction of whole rat brain was similar to that found for the crude mitochondrial fraction. The K_D values for (+)-[^{3}H]nicotine and (−)-[^{3}H]nicotine were 2.6×10^{-7} and 7×10^{-8} *M*, respectively.

Previous reports indicated that (−)-nicotine was pharmacologically 10–30 times more potent than (+)-nicotine (2, 3). However, the *in vitro* binding of (−)-[^{3}H]nicotine to rat brain tissue appeared to lack stereospecificity in that (+)- and (−)-nicotine were equally effective in displacing (−)-[^{3}H]nicotine (5). These studies suggested that the isomers differed in efficacy rather than affinity. However, the direct comparison of (+)- and (−)-[^{3}H]nicotine binding data in the present study showed that (+)-[^{3}-H]nicotine has approximately one-third the affinity of (−)-[^{3}H]nicotine. Differences in both binding affinity and efficacy apparently account for pharmacological stereospecificity. Differences in brain levels of the stereoisomers could also contribute to potency differences that are based on dose.

Distribution Studies—The time course of (+)- and (−)-[^{3}H-]nicotine in rat brain and plasma is shown in Fig. 1. Maximum plasma levels of (+)- and (−)-[^{3}H]nicotine were observed at 10 and 20 min, respectively. Maximum brain levels of both stereoisomers occurred at 20 min. The brain and plasma concentrations of (−)-[^{3}H]nicotine were significantly greater than those of (+)-[^{3}H]nicotine for almost all time points. The differences in brain levels of the two stereoisomers are probably not due to binding affinity for brain or to penetration of the blood brain barrier but rather to differences in plasma levels. Levels of total radioactivity in plasma did not differ between the (+)- and (−)-[^{3}H]nicotine-treated animals at any time point.

These data suggest that both isomers are absorbed at a similar rate following subcutaneous injection. It would appear that (+)-[^{3}H]nicotine is metabolized at a faster rate than (−)-[^{3}H]nicotine, although this point needs investigation. The brain levels of total radioactivity in the (−)-[^{3}H]nicotine-treated animals were twice as high as those in the (+)-[^{3}H]nicotine treatment group, reflecting the poor penetrability of nicotine metabolites into brain. The differences in the brain levels of (+)- and (−)-[^{3}H]nicotine show that potency ratios of the stereoisomers of nicotine should be based on concentration at the target tissue rather than on dose.

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[7] Model B, Beckman Instruments, Palo Alto, Calif.
[8] Triton X-100, New England Nuclear, Boston, Mass.
[9] Polytron, Brinkmann Instruments, Westbury, N.Y.