Improved Delivery Through Biological Membranes XIX: Novel Redox Carriers for Brain-Specific Chemical Delivery Systems

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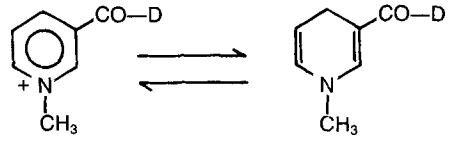
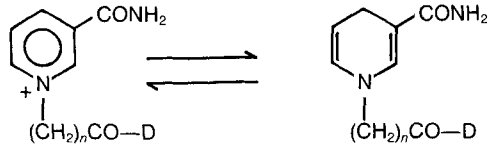
Abstract □ New dihydropyridine ⇌ pyridinium salt-type redox carrier systems were developed in which the drug is linked via the ring nitrogen atom of nicotinamide. The rate of oxidation of the dihydropyridine forms, and thus the overall and brain-specific distribution of the corresponding 3-carbamoyl-1-carbamoylalkyl-drug quaternary salts, depends on the number of methylene groups separating the ring nitrogen and the carbamoyl function linked to the drug.

The impermeability of the blood–brain barrier (BBB) to polar chemotherapeutic agents[1-4] prevents effective treatments of a number of cerebral diseases. We have reported a general method for providing brain-enhanced and/or specific, sustained delivery of a variety of drugs, using a dihydropyridine ⇌ pyridinium salt type redox carrier system.[5,6] The actual redox delivery system used for the delivery of phenylethylamine,[7] dopamine,[8,9] and for testosterone[10] was the trigonelline (**1**) ⇌ dihydrotrigonelline (**2**) type, connected to the drug (D) to be delivered via an amide or an ester bond, formed with the carboxy groups of trigonelline:



1 **2**

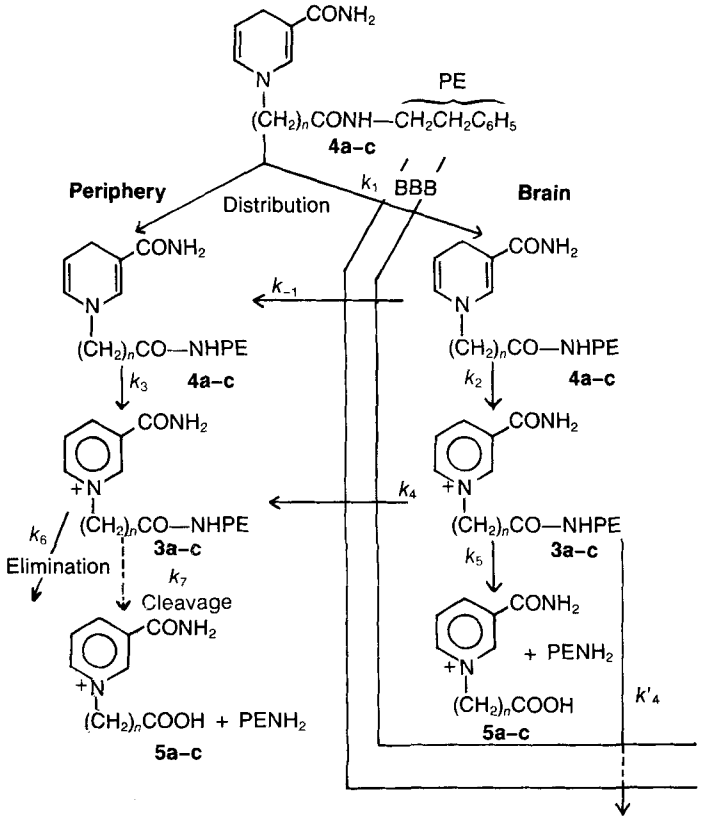
There are, however, many other analogous delivery systems which have to be studied, since the overall sequence of delivery–"lock-in"–sustained release of the drug involves many rate processes, which can only be optimized if an arsenal of delivery forms are developed. While the trigonelline-based carriers have the advantage of low toxicity, other redox pairs are even closer to the ubiquitous $NAD^+ \rightleftharpoons NADH$ coenzyme system. In one of these possible carriers, the drug is linked to the endocyclic nitrogen atom of nicotinamide, such as in **3** and **4**:



3 **4**

The rate of oxidation of the dihydro form and the rate of release of D is expected to depend on the number of methylene groups inserted (n), the electronic and steric properties of the D, and its link to the carrier.

The present paper describes our investigations on these type of carrier systems. In order to have a direct comparison to the trigonelline ⇌ dihydrotrigonelline carrier,[7] phenylethylamine was used again as a model compound (D). Scheme I illustrates the design delivery system. The essential features include the facile delivery of the lipophilic **4** to the CNS. Following oxidation of the carrier part, the corresponding hydrophilic quaternary salt(s) **3** formed will be excreted (k_6, k_7) while being "locked-in" the brain (k_5, k_4). Due to the large differences between the efflux rates from the brain of **4** (k_{-1}) and **3** (k_4), combined with the different oxidation rates (k_2, k_3), the concentration of **3** in the brain is expected to increase until all of the circulatory **4** is transformed into **3** or eliminated. The brain/blood concentration ratio of **3** is thus expected to increase steadily, reaching a maximum. Since **3** is still an inactive precursor for D, both systemic and central toxic effects are expected to be reduced. The facile elimination of **3** from the general circulatory system prevents the release of D in the periphery, while the high brain concentration of the inactive **3** will result in the sustained release of the D ultimately only in the brain.



*Scheme I—For **1a–5a**, n = 1; for **1b–5b**, n = 2; for **1c–5c**, n = 3.*

Experimental Section

Apparatus—Melting points were determined on a Mel-Temp apparatus and are uncorrected. Elemental analyses were

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performed at Atlantic Microlab, Inc., Atlanta, GA. IR spectra were determined with a Beckman Acculab 1 double-beam recording spectrophotometer. ^{1}H NMR spectra were taken on a Varian T60 instrument, except for using a Nicolet 300-MHz spectrometer for **4a**. All chemical shifts reported are in δ units (ppm) relative to tetramethylsilane. UV spectra were determined with a Cary model 210 spectrophotometer.

HPLC analyses were performed on a component system consisting of a Beckman model 110a solvent delivery system, model 210 injector, and model 153 UV detector operated at 254 nm. A 30-cm × 3.9-mm i.d. reversed-phase μ-Bondapak C_{18} column (Waters Associates), operated at ambient temperature, was used for all the separations. The mobile phase used for the separation of the dihydropyridines (**4a–c**) and their oxidation products (**3a–c**) consisted of a 0.001 M solution of pentane-1-sulfonate sodium (ALTEX in acetonitrile):0.01 M aqueous dibasic ammonium phosphate:tetrahydrofuran (1:3:0.2) at a flow rate of 3 mL/min. The retention times were: **4a**, 2.5 min; **4b**, 2.8 min; **4c**, 3.2 min; **3a**, 3.4 min; **3b**, 3.7 min; **3c**, 4.2 min.

***N*-(β-Phenethyl)-2-bromoacetamide (6a)**—To a stirred solution of 2.263 g (0.0187 mol) of phenethylamine in 10 mL of 8% sodium hydroxide solution, cooled to −10°C, was introduced in a dropwise manner 3.14 g (0.02 mol) bromoacetyl chloride. The mixture was stirred at <10°C for 1 h. The resulting precipitate was removed by filtration, washed thoroughly with cold water, dried, and recrystallized from chloroform to give 3.2 g of **6a** (71%), mp 60–61°C. IR (KBr): 3280 (NH) and 1675 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 7.2 (s, 5, C_6H_5), 3.8 (s, 2, CH_2Br), 3.5 (q, 2, CH_2NH), and 2.83 ppm (t, 2, CH_2Ph).

***N*-(β-Phenethyl)-3-bromopropionamide (6b)**—The title compound was prepared as **6a** using 3.42 g (0.02 mol) of 3-bromopropionyl chloride instead of bromoacetyl chloride. Recrystallization from aqueous ethanol gave 3.6 g of **6b** (85%), mp 69–70°C (lit.[11] mp 55°C). IR (KBr): 3315 (NH) and 1640 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 7.2 (s, 5, C_6H_5), 3.6 (q, 4, CH_2Br and CH_2NH), and 2.8 ppm (t, 4, J = 7 Hz, CH_2Ph and CH_2CO).

***N*-(β-Phenethyl)-4-bromobutyramide (6c)**—This compound was prepared as **6a** using 2.22 g (0.012 mol) of 4-bromobutyryl chloride. Recrystallization from aqueous ethanol gave 2.3 g of **6c** (60%), mp 62–63°C. IR (KBr): 3100 (NH) and 1635 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 7.2 (s, 5, C_6H_5), 3.43 (m, 4, CH_2Br and CH_2NH), 2.75 (t, 2, CH_2Ph), and 2.16 ppm (m, 4, $COCH_2CH_2$).

3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylmethyl]pyridinium Bromide (3a)—To a solution of 2.419 g (0.01 mol) of *N*-(β-phenethyl)-2-bromoacetamide (**6a**) in 30 mL of dry acetonitrile was added 1.22 g (0.01 mol) of nicotinamide. The mixture was refluxed until disappearance of the reactants (3–4 d, monitored by TLC). For TLC, plates of Silica Gel G and a system of chloroform:methanol (9:1) was used. The acetonitrile was removed under reduced pressure, and the residue was recrystallized from methanol:chloroform to yield 2.8 g of **3a** (77%), mp 178–180°C. UV λ_{max} (methanol): 265 nm; IR (KBr): 3380 (NH), 3250 (NH), 1690 (CO), and 1655 cm^{-1} (CO); ^{1}H NMR (D_2O): δ 9.2 (s, 1, C_2 pyridine H), 9.04–8.83 (m, 1, C_5 pyridine H), 7.2 (s, 5, C_6H_5), 5.35 (s, 2, N^+—CH_2), 3.6 (t, 2, CH_2NH), and 2.86 ppm (t, 2, CH_2Ph).

Anal.—Calc. for $C_{16}H_{18}BrN_3O_2 \cdot H_2O$ (M_r, 364.2): C, 50.27; H, 5.23; N, 10.98. Found: C, 50.25; H, 4.77; N, 10.62.

3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylethyl]pyridinium Bromide (3b)—This compound was prepared as **3a** using 1.22 g (0.010 mol) of *N*-(β-phenethyl)-3-bromopropionamide (**6b**) and nicotinamide. Recrystallization from ethanol:benzene gave 2.5 g of **3b** (66%), mp 120–122°C. UV λ_{max} (methanol): 266 nm; IR (KBr): 3280 (NH), 3130 (NH), 1695 (CO), and 1640 cm^{-1} (CO); ^{1}H NMR (D_2O): δ 9.2 (s, 1, C_2 pyridine H), 8.93–8.80 (m, 2, C_6 and C_4 pyridine H), 7.0 (s, 5, C_6H_5), 4.7 (t, 2, N^+—CH_2), 3.27 (t, 2, CH_2NH), and 3.0–2.4 ppm (m, 4, J = 7 Hz, CH_2Ph and CH_2CO).

Anal.—Calc. for $C_{17}H_{20}BrN_3O_2$ (M_r, 378.3): C, 53.98; H, 5.33; N, 11.11. Found: C, 53.87; H, 5.35; N, 11.10.

3-Carbamoyl-1-[*N*-[β-[(phenyl)ethyl]carbamoylpropyl]pyridinium Bromide (3c)—This compound was prepared as **3a** using 4.05 g (0.015 mol) of *N*-(β-phenethyl)-4-bromobutyramide (**6c**) and nicotinamide. Recrystallization from ethanol:acetone gave 4.92 g of **3c** (83%), mp 112–114°C. UV λ_{max} (methanol): 265 nm; IR (KBr): 3350 (NH), 3320 (NH), 1692 (CO), and 1642 cm^{-1} (CO); ^{1}H NMR (D_2O): δ 9.27 (s, 1, C_2 pyridine H), 9.07–8.70 (m, 2, C_6 and C_4 pyridine H), 8.27–7.87 (m, 1, C_5 pyridine H), 7.25 (s, 5, C_6H_5), 4.47 (t, 2, N—CH_2), and 3.57–2.20 ppm (m, 8, CH_2NH, CH_2Ph, and $COCH_2CH_2$).

Anal.—Calc. for $C_{18}H_{22}BrN_3O_2$ (M_r, 392.3): C, 55.11; H, 5.65; N, 10.71. Found: C, 54.93; H, 5.67; N, 10.70.

3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylmethyl]-1,4-dihydropyridine (4a)—To a solution of 3.64 g (0.01 mol) of **3a** in 150 mL of deaerated 15% aqueous methanol were added 5.04 g (0.06 mol) of sodium bicarbonate. The mixture was stirred in an ice bath, and 6.96 g (0.04 mol) of sodium dithionite was added over a period of 5 min. The mixture was stirred for 1 h under nitrogen, a pale-yellow crystalline precipitate was formed. The precipitate was removed by filtration, washed with water, and recrystallized from aqueous methanol to give 2.4 g of **4a** (84%), mp 126–128°C. UV λ_{max} (methanol): 348 nm; IR (KBr): 3280 (NH), 1680 (CO), and 1645 cm^{-1} (CO); ^{1}H NMR (acetone-d_6): δ 7.51–7.20 (m, 5, C_6H_5), 7.02 (s, 1, C_2 pyridine H), 6.28–6.18 (s, 2, $CONH_2$), 5.92–5.87 (m, 1, C_6 pyridine H), 4.81–4.74 (m, 1, C_5 pyridine H), 3.87 (s, 2, NCH_2CO), 3.59–3.50 (m, 2, CH_2NH), 3.25–3.15 (m, 2, C_4 pyridine H), and 2.94–2.87 ppm (t, 2, CH_2Ph).

Anal.—Calc. for $C_{16}H_{19}N_3O_2 \cdot 0.75\ H_2O$ (M_r, 285.3): C, 64.30; H, 6.91; N, 14.06. Found: C, 64.32; H, 6.91; N, 14.06.

3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylethyl]-1,4-dihydropyridine (4b)—As described above, **3b** (3.78 g, 0.01 mol) was reduced with sodium dithionite (6.96 g, 0.04 mol). After completion of the reaction, **4b** was extracted with ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. A yield of 2.4 g (80%) of **4b** was obtained as a yellowish amorphous powder, mp 121–123°C. UV λ_{max} (methanol): 350 nm; IR (KBr): 3430 (NH), 3260 (NH), 1670 (CO), and 1630 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 7.26 (s, 5, C_6H_5), 7.05 (s, 1, C_2 pyridine H), 6.83–6.46 (m, 1, C_6 pyridine H), 5.81 (br s, 2, $CONH_2$), 5.68 (br s, 1, CONH), 4.85–4.50 (m, 1, C_5 pyridine H), 3.60–3.18 (t, 6, NCH_2, CH_2NH, and C_4 pyridine H), 2.93–2.56 (t, 2, CH_2Ph), and 2.46–2.16 ppm (t, 2, CH_2CO).

Anal.—Calc. for $C_{17}H_{21}N_3O_2 \cdot 0.75\ H_2O$ (M_r, 299.4): C, 65.26; H, 7.24; N, 13.43. Found: C, 65.19, H, 6.87; N, 13.61.

3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylpropyl]-1,4-dihydropyridine (4c)—As described above for the preparation of **4b, 3c** (3.92 g, 0.01 mol) was reduced with sodium dithionite (6.96 g, 0.04 mol). A yield of 2.2 g (65%) of **4c** was obtained as an orange-yellow amorphous powder, mp 55–60°C. UV λ_{max} (methanol): 358 nm; IR ($CHCl_3$): 3325 (NH), 1682 (CO), and 1645 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 7.2 (s, 5, C_6H_5), 6.96 (s, 1, C_2 pyridine H), 6.76–6.46 (m, 1, C_6 pyridine H), 5.9 (br s, 2, $CONH_2$), 5.78–5.53 (d, 1, CONH), 5.00–4.53 (m, 1, C_5 pyridine H), 3.56–3.26 (t, 2, CH_2NH), 3.23–2.60 (m, 6, NCH_2, C_4 pyridine H, and CH_2Ph), and 2.3–1.6 ppm (m, 4, $COCH_2CH_2$).

Anal.—Calc. for $C_{18}H_{23}N_3O_2 \cdot 0.75\ H_2O$ (M_r, 313.4): C, 66.07; H, 7.49; N, 12.85. Found: C, 66.05; H, 7.56; N, 12.84.

Oxidation of 4a–c to 3a–c—*With Silver Nitrate*—To 5 mL of saturated methanolic $AgNO_3$ solution was added 1 mL of a 5% methanolic solution of the appropriate dihydropyridine derivative **4a, 4b,** or **4c.** The mixture was shaken and centrifuged after 5, 10, 20, or 30 min at room temperature or at the boiling temperature of methanol, and the supernatant was

analyzed. Disappearance of the absorption at ~350 nm due to the dihydropyridine and appearance of the absorption at ~260 nm characteristic of the quaternary salt confirmed the oxidation. Additional tests with silver nitrate were carried out in buffer solutions of pH 5.0, 7.0, 7.4, 8.0, and 10.0.

With Hydrogen Peroxide—To 10 mL of 30% H_2O_2 solution was added 1 mL of a 5% methanolic solution of the appropriate dihydro derivative (**4a, 4b,** or **4c**). The mixture was allowed to stand for 5, 10, 20, or 30 min either at the ambient or at the boiling temperature, and it was analyzed for **3a–c** as in the previous case. The studies were repeated in the presence of 0.1 mL of ammoniated CuO, as a source of Cu^{2+}.

In Vitro Oxidation of the Dihydro Derivatives 4a–c—*In Human Plasma*—The freshly collected plasma used was obtained at the Civitan Regional Blood Center, Inc. (Gainesville, FL) and contained ~80% plasma diluted with citrate phosphate dextrose adenine solution USP as anticoagulant. The plasma was stored in a refrigerator and used the next day. Two-hundred microliters of the freshly prepared 0.61 M solution of the appropriate dihydropyridine derivative (**4**) in methanol 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 1.22×10^{-2} mol/L. Samples of 0.5 mL were withdrawn from the test medium every 10 min, added immediately to 3.5 mL of ice-cold acetonitrile, shaken vigorously, and placed in a freezer. When all samples had been collected, they were centrifuged; the supernatants were filtered through nylon 66 filters and analyzed in duplicate by HPLC.

In Human Blood—The freshly collected blood used was obtained at the Civitan Regional Blood Center, Inc. (Gainesville, FL) and contained citrate phosphate dextrose adenine solution USP as the anticoagulant. The blood was stored in a refrigerator and used the next day. One-hundred microliters of a freshly prepared 0.18 M solution of the appropriate dihydro compound **4a, 4b,** or **4c** in methanol was added to 10 mL of blood, previously equilibrated to 37°C in a water bath, and mixed thoroughly to result in an initial concentration of 1.8×10^{-3} mol/L. Samples of 0.5 mL were withdrawn from the test medium every 10 min and worked up as above.

In Beef Liver Microsomes—The beef liver microsomes used were obtained from the laboratories of Drs. Charles Allen and T. W. O'Brien, Department of Biochemistry, University of Florida (Gainesville, FL). The microsomes (2 g) were homogenized in 20 mL of aqueous 0.11 M phosphate buffer, pH 7.4. One-hundred microliters of a freshly prepared 0.1 M solution of the dihydro compound **4a** in methanol was added to 10 mL of the beef liver microsome homogenate, previously equilibrated to 37°C in a water bath, and mixed thoroughly to result in an initial concentration of 1×10^{-3} mol/L. Samples of 0.5 mL were withdrawn from the test medium every 10 min and worked up as above.

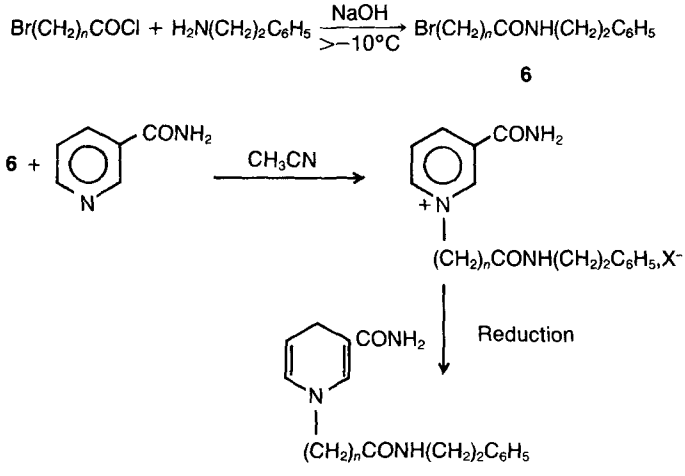
In Rat Brain Homogenates—A 20% rat brain homogenate of Sprague–Dawley rats was freshly prepared in aqueous 0.11 M phosphate buffer, pH 7.4. One-hundred microliters of a freshly prepared 0.18 M solution of the dihydro compound **4a, 4b,** or **4c** in methanol was added to 10 mL of the 20% rat brain homogenate, previously equilibrated to 37°C in a water bath, and mixed thoroughly to result in an initial concentration of 1.8×10^{-3} mol/L. Samples of 0.5 mL were withdrawn from the test medium every 10 min and worked up as above.

Kinetics of the Disappearance of the Quaternary Salts (3) from the Brain Homogenate—Freshly perfused Sprague–Dawley rats brains (4.0 g) were homogenized in 20 mL of phosphate buffer, pH 7.4. Two-hundred microliters of the freshly prepared 0.024 M solution of the appropriate pyridinium bromide derivative **3a, 3b,** or **3c** in aqueous methanol (1:1) was added to 20 mL of the 20% rat brain homogenate, 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. At each time period, 1 mL of the test medium was removed, shaken thoroughly with 4 mL of ice-cold acetonitrile, and centrifuged; the supernatant was filtered through a nylon 66 filter and analyzed in duplicate by HPLC. The amount of the quaternary compound in the sample was determined from a standard calibration curve and percentage recovery of a sample taken at time zero.

In Vivo Delivery Study of 3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylalkyl-1,4-dihydropyridines (4a–c)—A group of 25 male Sprague–Dawley rats (except for **4a**, which was studied in female rats) of average weight of 270–300 g was anesthetized with droperidol–fentanyl citrate (Inovar), and the appropriate freshly prepared dihydro compound (**4a, 4b,** or **4c**) was injected through the external jugular vein as a solution in Me_2SO (0.25 g/mL) at a dose level of 125 mg/kg of body weight. (The amount of Me_2SO thus injected is <0.2 g/kg, far below the dose of 1 g/kg that was shown[12] as still not affecting the permeability of the blood–brain barrier.) At appropriate time periods, 2 mL of blood was withdrawn from the heart and added immediately with a tared tube containing 8 mL of acetonitrile, which was afterwards weighed to determine the amount of the blood added. The animal was then perfused with 20 mL of saline solution and decapitated, and the brain, liver, kidneys, and testes were collected. The organs obtained were weighed and, together with the blood samples, immediately placed in the freezer for overnight storage. Whole brain, kidneys, and testes and 2 g of liver were each homogenized in 2 mL of water; 8 mL of acetonitrile was added, and the mixture was homogenized again and centrifuged. The supernatants from the organs and the blood samples were filtered through nylon 66 filters and analyzed in duplicate for the corresponding quaternary compounds (**3a–c**) by HPLC. Quantitation was done by using recovery standard curves obtained by introducing a known amount of the appropriate salt (**3a, 3b,** or **3c**) in either blood, brain, kidney, liver, or testes homogenate and then treating them in the same manner as mentioned above.

Results and Discussion

The 1,4-dihydropyridine derivatives **4** were synthesized according to Scheme II. The synthesis of the pyridinium bromides **3** was achieved by coupling phenethylamine with the bromoacyl chlorides[11] to give the *N*-(β-phenethyl) bromoamides **6** which were treated with nicotinamide in dry acetonitrile, in analogy to a reported procedure.[13]



Scheme II—*For* ***1a–5a***, n = *1; for* ***1b–5b***, n = *2; for* ***1c–5c***, n = *3.*

Attempts to prepare the quaternary salts **3** directly either by coupling of acids **5** with phenethylamine using dicyclo-

Table I—Rates of Oxidative Conversion in Biological Fluids[a] of 3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylalkyl]-1,4-dihydropyridines (4a–c) to the Corresponding Quaternary Pyridinium Salts (3a–c)[b]

Medium	4a → 3a				4b → 3b				4c → 3c			
	n	$k \times 10^{-3}$, min^{-1}	r	$t_{1/2}$, min	n	$k \times 10^{-3}$, min^{-1}	r	$t_{1/2}$, min	n	$k \times 10^{-3}$, min^{-1}	r	$t_{1/2}$, min
Brain homogenate	5	5.2668	0.9974	131.6	6	25.848	0.9876	26.8	7	36.587	0.9769	18.9
Whole blood	6	2.5743	0.9965	269.2	5	9.7143	0.9570	71.3	6	10.070	0.9867	68.8
Human plasma	6	2.4147	0.9723	287.0	—	—	—	—	—	—	—	—
Liver microsome homogenate	5	29.036	0.9785	23.8	—	—	—	—	—	—	—	—

[a] At 37°C, undiluted citrated human blood, 80% fresh human plasma, 20% rat brain, and 10% beef liver microsome homogenates were used. The conversions of **4a** to **3a**, **4b** to **3b**, and **4c** to **3c** followed the changes in their characteristic retention times, against appropriate reference samples. [b] The dihydro trigonelline analogue (ref. 7), showed half-lives of oxidation of 28.2, 13.7, 64.2, and 14.4 min in brain homogenate, whole blood, human plasma, and rat liver homogenate, respectively.

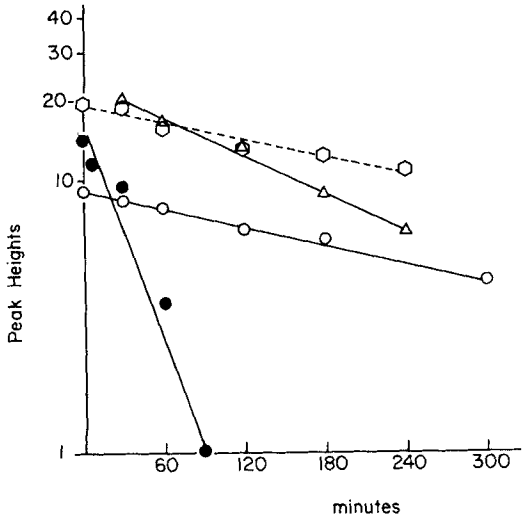


Figure 1—*Semilogarithmic plot of peak heights of 3-carbamoyl-1-[N-[β-(phenyl)ethyl]carbamoylmethyl]-1,4-dihydropyridine (**4a**) against time in brain homogenate (△), whole blood (—○—), plasma (--⬡--), and liver microsome homogenate (●).*

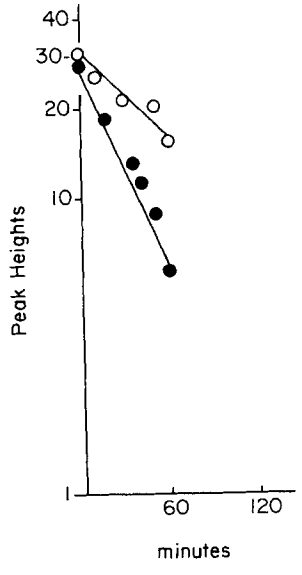


Figure 2—*Semilogarithmic plot of peak heights of 3-carbamoyl-1-[N-[β-(phenyl)ethyl]carbamoylethyl]-1,4-dihydropyridine (**4b**) against time in brain homogenate (●) and whole blood (○).*

hexylcarbodiimide in pyridine or 2-ethoxy-1(2*H*)-quinolinecarboxylic acid ethyl ester in pyridine or dry ethanol were unsuccessful, due to low solubility of acids **5** in the mixture and because of their reduced electrophilicity caused by the zwitterionic form.

The 1,4-dihydropyridine derivatives **4** were prepared by reduction of the quaternary salts **3** using the selective and ambivalent[15] reducing agent sodium dithionite, in a slightly alkaline medium. It was reported that reduction of the 3-substituted pyridinium salts with sodium dithionite in mildy basic solutions affords exclusively the corresponding 1,4-dihydropyridines.[16] Indeed, **4a–c**, based on their spectral (UV and ^{1}H NMR) and chemical properties[13,15-17] were all confirmed to have the 1,4-dihydro forms. These dihydro derivatives were found to be relatively stable, in comparison with the reported 1-methyl-3-(*N*-phenethylcarbamoyl)-1,4-dihydropyridine (**7**);[7] however they can be quantitatively oxidized back to the corresponding quaternary salts by H_2O_2 in the presence of a catalytic amount of Cu^{2+} (based on spectral data and characterization of the isolated products). Contrary to **7**, **4a–c** could not be easily oxidized to **3a–c** with methanolic $AgNO_3$.

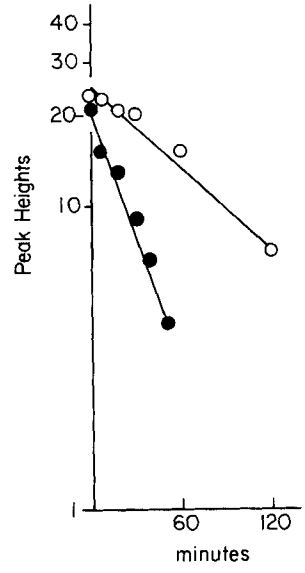


Figure 3—*Semilogarithmic plot of peak heights of 3-carbamoyl-1-[N-[β-(phenyl)ethyl]carbamoylpropyl]-1,4-dihydropyridine (**4c**) against time in brain homogenate (●) and whole blood (○).*

The in vitro kinetics in biological fluids indicated facile oxidative conversion of the dihydro derivatives **4b** and **4c** to the corresponding quaternary salts **3b** and **3c**, as indicated in Table I and Figs. 1–3. Compound **4a** (Table I) appeared to be significantly more stable under these conditions. This increased stability of the dihydro form can be attributed to the close proximity of the electron-withdrawing amido group. It was found that the quaternary salts **3a**, **3b**, and **3c** are stable toward hydrolysis in brain homogenate.

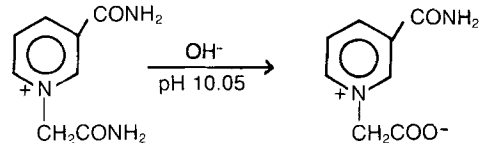
The results of the in vivo studies (Table II) strongly support the concept shown in Scheme I. Thus, after one injection of the dihydropyridine derivatives (**4a–c**) to rats, the quaternary salts (**3a–c**) disappear quickly from the blood, while relatively high levels are maintained in the brain. Of the three systems studied, the three methylene-separated **4c** ⇌ **3c** provides the best results, in terms of sustained, specific brain delivery. While

Table II—Concentrations Against Time of 3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylalkyl]pyridinium Cations in Brain, Blood, Kidney, Liver, and Testes after Administration of 3-Carbamoyl-1-[*N*-[β-(phenyl)ethyl]carbamoylalkyl]-1,4-dihydropyridines

Time, min	Organ	Concentration ± SEM, μg/g of Blood or Wet Organ		
		3a	**3b**	**3c**
5	Brain	11.95 ± 1.14	9.93 ± 0.65	24.28 ± 2.06
	Blood	78.25 ± 2.60	99.46 ± 3.37	155.20 ± 2.29
	Kidney	167.47 ± 16.75	238.44 ± 15.49	440.14 ± 2.07
	Liver	—[a]	9.92 ± 1.81	65.09 ± 0.92
	Testes	—[b]	5.86 ± 1.29	8.18 ± 0.50
30	Brain	3.20 ± 0.34	6.20 ± 1.13	19.57 ± 1.25
	Blood	5.15 ± 0.29	6.10 ± 0.84	6.99 ± 1.35
	Kidney	47.70 ± 1.67	64.20 ± 1.86	196.53 ± 50.01
	Liver	—[a]	—[a]	103.14 ± 36.47
	Testes	—[b]	4.21 ± 0.29	8.76 ± 1.05
60	Brain	4.79 ± 0.18	13.36 ± 0.92	14.56 ± 1.22
	Blood	3.23 ± 1.82	5.19 ± 0.55	—[a]
	Kidney	30.43 ± 11.49	376.95 ± 17.23	84.11 ± 9.98
	Liver	1.36 ± 1.60	14.33 ± 3.97	7.73 ± 1.18
	Testes	—[b]	5.03 ± 1.06	6.36 ± 0.75
120	Brain	6.64 ± 0.57	1.28 ± 0.008	21.06 ± 1.56
	Blood	1.77 ± 0.15	1.37 ± 0.22	—[a]
	Kidney	21.43 ± 0.15	30.77 ± 0.95	111.39 ± 31.24
	Liver	—[a]	—[a]	13.16 ± 21.11
	Testes	—[b]	—[a]	4.44 ± 0.39

[a] Below detection limit. [b] Female.

the maximum brain concentrations are lower than in the case of the trigonelline-based system **7**, the brain concentrations are clearly maintained at a high level throughout the experiment (2 h). Contrary to the analogous trigonelline–phenylethylamine system,[7] the amides of the type **3** seem to be stable in brain homogenate and, thus, did not release phenylethylamine in any appreciable amount within several hours. It is expected, however, to see some amide cleavage, especially in vivo, since it was shown[13] that the simple amide **8** is hydrolyzed, although slowly, to **9**:



8 **9**

In addition, recent data[18] indicate facile electrochemical cleavage (at pH 7.4, 1.0-V potential difference) of amines from analogous quaternary pyridinium amides. It was suggested[18] that similar potential difference triggers neurotransmitter release at the synapses.

References and Notes

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