Improved Delivery through Biological Membranes. 13.[1] Brain-Specific Delivery of Dopamine with a Dihydropyridine ⇌ Pyridinium Salt Type Redox Delivery System

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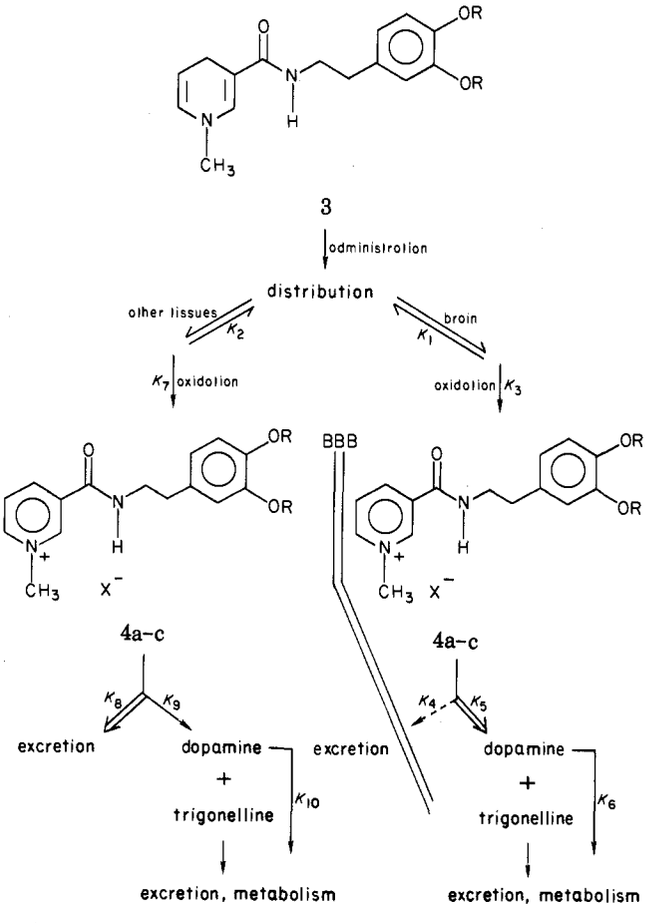
The dihydropyridine ⇌ pyridinium salt type redox delivery system was used for the brain-specific delivery of dopamine. In vivo administration of the catechol-protected dopamine coupled with 1,4-dihydrotrigonelline as the carrier resulted in brain-specific, high and sustained concentrations of the 1-methyl-3-[*N*-[β-(3,4-dihydroxyphenyl)ethyl]carbamoyl]pyridinium salt, the direct dopamine precursor, locked in the brain for many hours, while systemic concentration decreased fast, with a $t_{1/2}$ of less than 30 min. Significant dopaminergic activity was observed in the brain, which was sustained for hours.

Parkinsonism, a striatal dopamine deficiency syndrome,[3] cannot be treated directly with dopamine. Dopamine and related catecholamines evidently do not cross the blood–brain barrier (BBB).[4] L-Dopa, which could be considered as a prodrug for dopamine, was first discovered to be useful for the treatment of Parkinsonism more than 20 years ago.[5,6] Now L-Dopa is generally the best available treatment but, unfortunately, at the cost of many undesirable side effects.[7] Its peripheral side effects, which range from nausea and vomiting to cardiac arrythmias and hypotension, appear to be due to one or more of its metabolic products rather than L-Dopa per se. L-Aromatic amino acid decarboxylase is responsible for the major metabolism of L-Dopa whether prior, during, or after absorption. Concurrent administration of L-Dopa with an inhibitor of aromatic amino acid decarboxylase, which should not be able to penetrate the BBB, reduces the decarboxylation of L-Dopa in peripheral tissues. Such reduction allows higher proportions of L-Dopa to reach the CNS and at the same time diminishes the peripheral side effects considerably, particularly vomiting and cardiac arrythmias, but a number of serious side effects still persist.[7,8] Attempts were also made to alleviate the well-known dissolution, absorption, and metabolism problems of L-Dopa,[9-12] by using prodrug approaches.[13,14]

It would be still most desirable to deliver dopamine directly to the brain, and specifically to the brain, in a sustained manner.

This report describes the application of our novel chemical-delivery system,[15] based on a pyridinium ⇌ dihydropyridine redox carrier, for brain-specific delivery of dopamine.

Scheme I[a]



[a] a, R = H; b, R = $COCH_3$; R = $CO(CH_3)_5$.

This redox delivery system was successfully applied for brain-specific delivery[16] of phenylethylamine, by using the dihydro derivative 1 to deliver quaternary precursor 2, which while "locked in" the brain provided a sustained release form of phenylethylamine:

The object of the present work is the sustained delivery of dopamine to the brain in pharmacologically active concentrations paralleled with much lower concentrations in the peripheral circulation and other tissues. According to Scheme I, the designed delivery system for dopamine, compound 3, on administration is expected to be distributed throughout the body and, due to its lipophilic character, will penetrate quite easily the blood–brain barrier and enter the CNS. Following oxidation both in the brain

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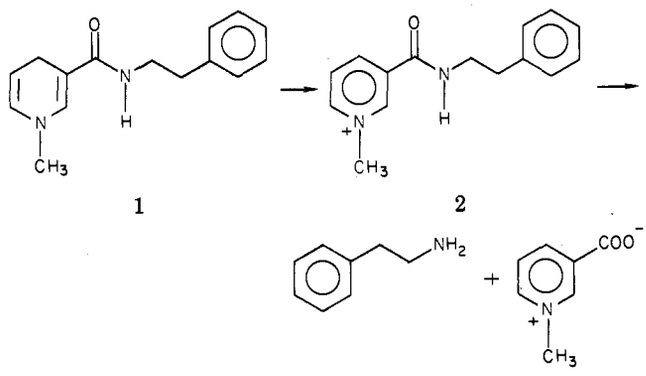
(2) On leave of absence from the Faculty of Pharmacy, University of Assiut, Egypt.
(3) H. Ehringer and O. Hornykiewicz, *Klin. Wochenschr.*, **38**, 1236 (1960).
(4) B. E. Roos and G. Steg, *Life Sci.*, **3**, 351 (1964).
(5) A. Barbeau, *Excepta Med., Int. Congr. Ser.*, **38**, 152 (1961).
(6) W. Birkmayer and O. Hornykiewicz, *Wien. Klin. Wochnenschr.*, **73**, 787 (1961).
(7) A. Barbeau, *Trends Pharmacol. Sci.*, **2**(11), 297 (1981).
(8) A. Barbeau and M. Roy, *Neurology*, **26**, 399 (1976).
(9) H. Ninterberger, *Biochem. Med.*, **5**, 412 (1971).
(10) H. Shindo, T. Komai, K. Tanaka, E. Nakajima, and N. Miyakoshi, *Chem. Pharm. Bull.*, **21**, 826 (1973).
(11) C. O. Rutledge and M. M. Hoehn, *Nature (London)*, **244**, 447 (1973).
(12) R. L. Bronaugh, R. J. McMurtry, M. M. Hoehn, and C. O. Rutledge, *Biochem. Pharmacol.*, **24**, 1317 (1975).
(13) N. Bodor, K. B. Sloan, T. Higuchi, and K. Sasahara, *J. Med. Chem.*, **20**, 1435 (1977).
(14) A. M. Felix, D. P. Winter, S. S. Wang, I. D. Kulesha, W. R. Pool, D. L. Hane, and H. Sheppard, *J. Med. Chem.*, **17**, 422 (1974).
(15) N. Bodor, H. H. Farag, and M. E. Brewster, *Science*, **214**, 1370 (1981).
(16) N. Bodor and H. H. Farag, *J. Med. Chem.*, **26**, 313 (1983).

0022-2623/83/1826-0528$01.50/0



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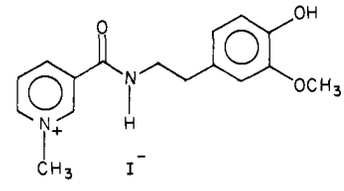
and in the other tissues, the corresponding hydrophilic quaternary salt (4) is formed. The quaternary salt 4 will be essentially "locked in" the brain, and its concentration is expected to increase with time until reaching a maximum, which depends primarily on the relative rates of entrance of the dihydro compound (3) to the brain (K_1) as compared to K_2, the rate of oxidation of the dihydro form to the quaternary (K_3 and K_7) and the rates of its disappearance from the brain ($K_4 + K_5$). At the same time, the very water-soluble quaternary form(s) 4 is expected to be excreted readily via the kidney and the liver ($K_8 \gg K_4$). Derivatives 4 are still essentially inactive forms ($K_8 \gg K_9$); thus, systemic activity–toxicity should be minimized. Hence, the concentration of 3 and 4 in the blood is expected to decrease fast. The ratio of the quaternary salt 4 in the brain relative to the blood is expected to be increasing to the point where 4 can only be found in the brain. The quaternary 4, whether in the brain, blood, or other tissues, is expected to release dopamine and trigonelline, depending on the rates of site-specific conversion of the precursor 4 to the drug at each of these sites. The concentration of the released dopamine at any time is expected to be much higher in the brain than in the blood or other tissues. Also, as the enzymatic transformation of the quaternary precursor 4 to the drug (dopamine) is expected to be relatively slow, it will allow a sustained release of dopamine. Similarly to previous studies,[13] the simultaneous protection–lipophilic derivatization of the catechol system in dopamine was investigated (R other than H).

The 1,4-dihydropyridine derivatives (3) were prepared according to Scheme II.

In order to investigate whether any biotransformation of the free catechol is taking place by COMT, either before or after oxidation, the possible *O*-methyl metabolites (7 and 8) were synthesized separately following Scheme II with 3-methoxytyramine hydrochloride as the starting material.



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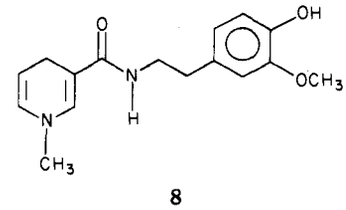

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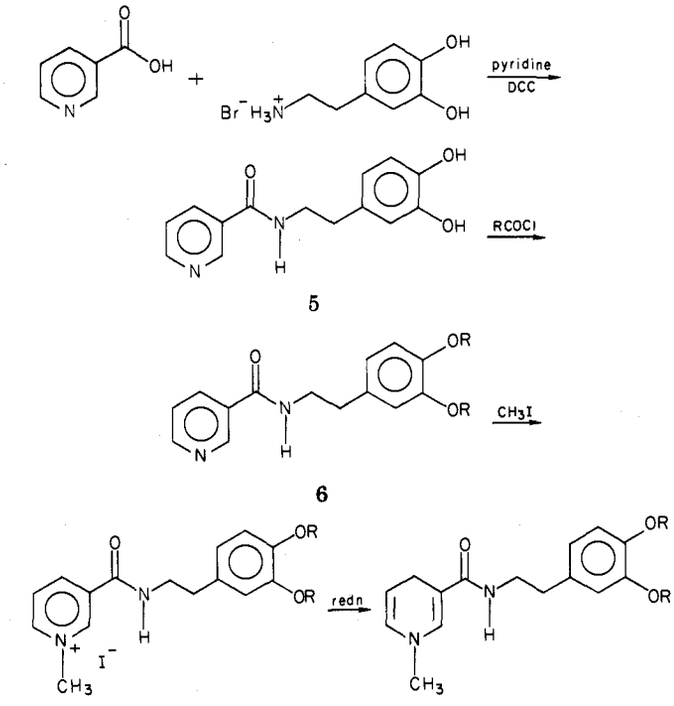
The stability of the 1,4-dihydropyridine derivatives (3) was investigated in the presence of the oxidizing agents,

Scheme II[a]



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alcoholic $AgNO_3$ and hydrogen peroxide. The in vitro rates of oxidation of the 1,4-dihydropyridine derivative (3c) in 80% plasma, 20% brain homogenate, 20% liver homogenate, and in whole blood were determined.

The dihydropyridine derivative (3c) was chosen for the in vivo study. A solution in Me_2SO was injected through the jugular vein to a group of male Sprague–Dawley rats, which were then sacrificed at various time intervals; their blood and brains were analyzed for the quaternary precursor of dopamine (4a). The in vivo dopaminergic activities of the selected compounds, 3c vs. 4a, were then determined.

Results and Discussion

N-Nicotinoyldopamine (5) could be obtained in good yields by coupling dopamine hydrobromide with nicotinic acid in pyridine as a solvent and with dicyclohexylcarbodiimide as the coupling agent. Attempts to prepare 5 by using dopamine free base were unsuccessful. This was also observed in the phenethylamine case.[16] As for the catechol protecting groups, the acetyl and pivalyl moieties were chosen due to their rather different steric and partitioning parameters. Acylation could be accomplished with the acyl chlorides by using conventional methods. Reduction of the quaternaries (4a–c and 7) has been accomplished by using sodium dithionite in mildly basic aqueous solution ($NaHCO_3$). It was observed that the dihydro compound obtained in the case of the quaternary 4b gives a faint green color with ferric ions, indicating partial hydrolysis of at least one of the acetyl moieties during reduction, even in the cold, weakly basic solution used as a medium. The dihydropyridine derivatives isolated (3a–c and 8) were assigned the expected 1,4-dihydropyridine structure, based on their NMR and UV spectra. Attempts to prepare the β-protonated enamine salts of the isolated dihydro derivatives were unsuccessful, due to acid-catalyzed addition reactions.[17] The 1,4-dihydropyridine derivatives (3a–c) were found to be relatively stable toward oxidation.

(17) N. Eisner and J. Kuthan, *Chem. Rev.*, **72**, 1 (1972).

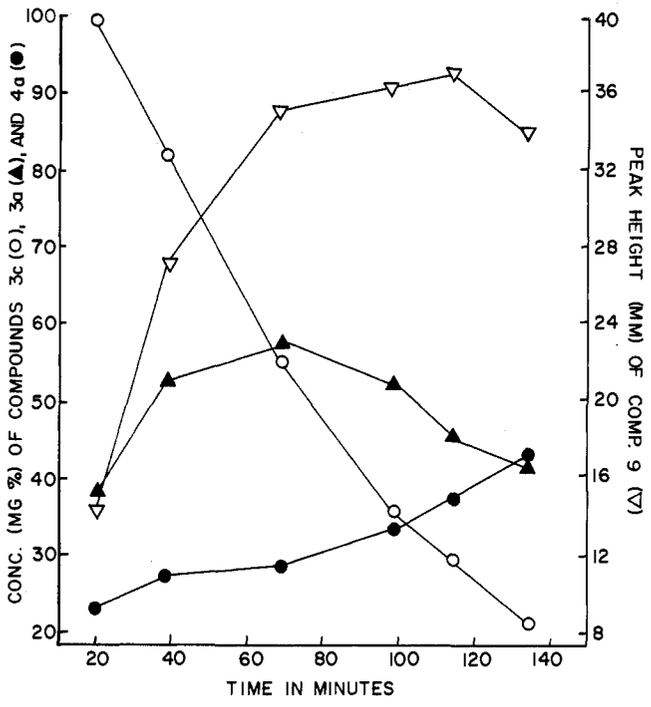


Figure 1. Time course of 1-methyl-3-[*N*-[β-[3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-1,4-dihydropyridine (**3c**, O) and its products, pivalyl dihydro derivative **9** (▽), the dihydrodopamine derivative **3a** (▲), and the quaternary dopamine precursor **4a** (●) in plasma.

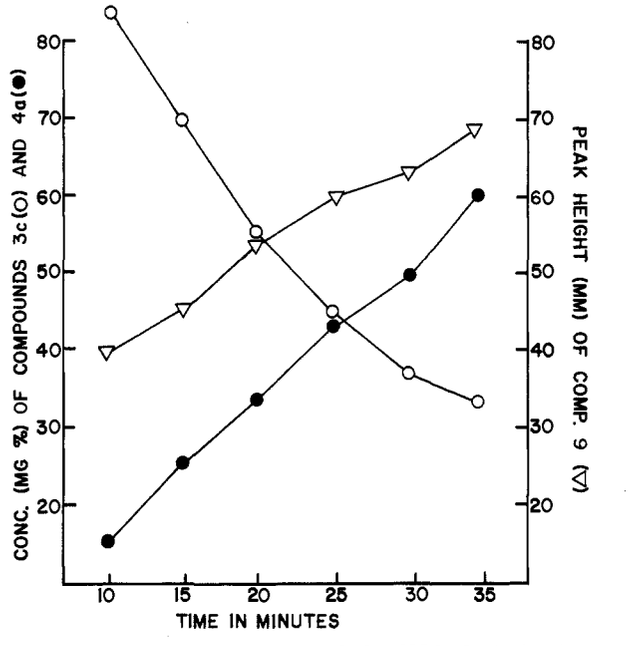


Figure 2. Time course of 1-methyl-3-[*N*-[β-[3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-1,4-dihydropyridine (**3c**, O) and its products, pivalyl dihydro derivative **9** (▽) and the quaternary dopamine precursor **4a** (●) in whole blood.

Compound **3c** could be quantitatively oxidized to the corresponding quaternary salt **4c** by H_2O_2 or alcoholic $AgNO_3$ solution as its analogue previously studied.[16]

In Vitro Studies. The diacetyl derivatives (**3b** and **4b**) as the analogous Dopa derivatives[13] appeared to be labile to hydrolysis and, therefore, were not used in the present investigation as potential brain specific delivery systems. The dipivalyl dihydro derivative (**3c**) was thoroughly investigated for its in vitro rates of disappearance and metabolic degradation in various biological fluids. It is evident that **3c** represents a rather complex case, as besides

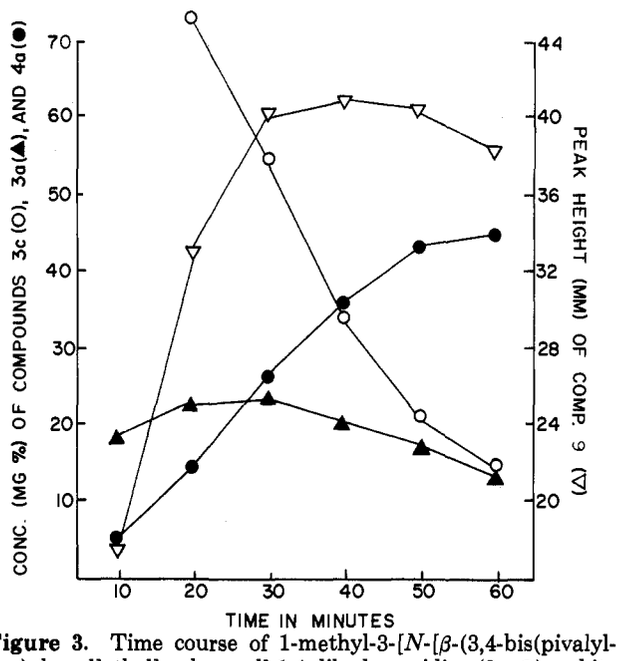


Figure 3. Time course of 1-methyl-3-[*N*-[β-(3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-1,4-dihydropyridine (**3c**, O) and its products, pivalyl dihydro derivative **9** (▽), the dihydrodopamine derivative **3a** (▲), and the quaternary dopamine precursor **4a** (●) in 20% brain homogenate.

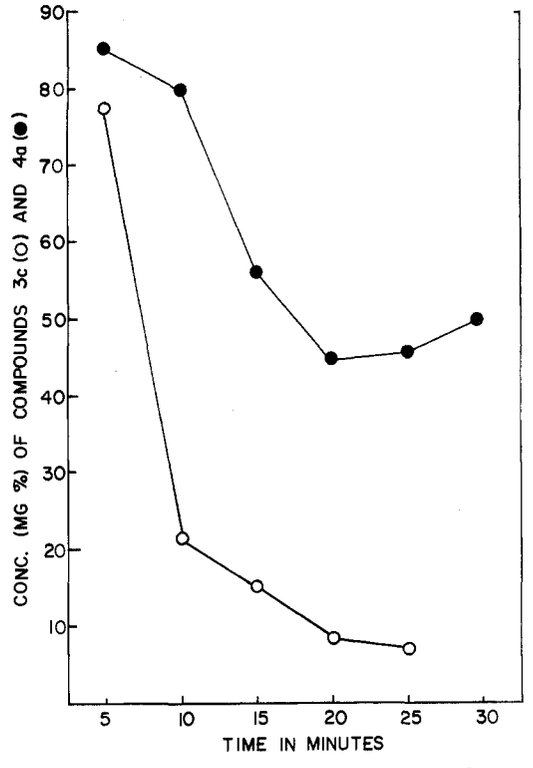
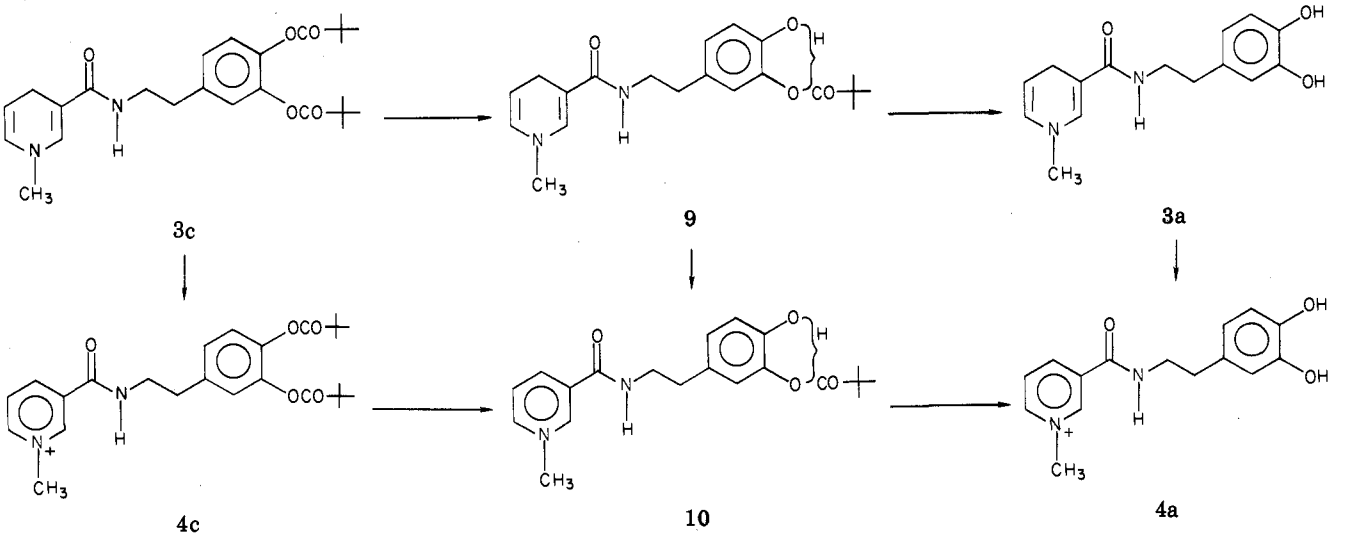


Figure 4. Time course of 1-methyl-3-[*N*-[β-[3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-1,4-dihydropyridine (**3c**, O) and its product, the quaternary dopamine precursor **4a** (●) in 20% liver homogenate.

oxidation, a two-step hydrolysis will also take place. Scheme III shows the interconversion of the possible components.

Figures 1–4 show the results of such an investigation. The apparent half-lives for the disappearance of **3c** in biological fluids at 37 °C were calculated. Although the process does not truly follow first-order kinetics, the data fit very closely a pseudo-first-order process (Figure 5). The obtained values, 51 (80% plasma), 17 (20% brain homogenate), 18 (whole citrated blood), and 6 min (20%

Scheme III



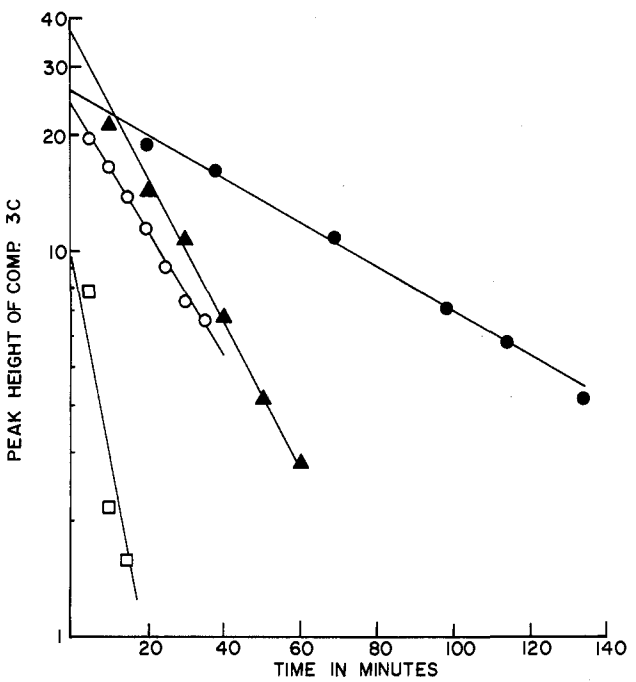


Figure 5. Semilog plot of peak heights of 1-methyl-3-[*N*-[β-(3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-1,4-dihydropyridine (**3c**) against time in plasma (●), brain homogenate (▲), whole blood (○), and liver homogenate (□).

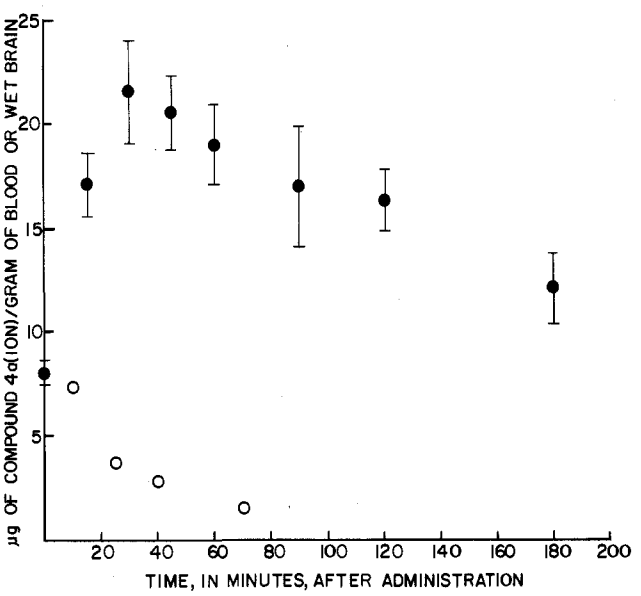


Figure 6. Concentrations against time of 1-methyl-3-[*N*-[β-(3,4-dihydroxyphenyl)ethyl]carbamoyl]pyridinium cation (**4a**) in brain (●) and in blood (○) following administration of 1-methyl-3-[*N*-[β-[3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-1,4-dihydropyridine (**3c**). The error bars indicate SEM.

liver homogenate), indicate a reasonable stability of the dihydro derivative **3c**. The values are comparable to the corresponding ones obtained for the analogous noncatechol derivative.[16] The disappearance of **3c** is accompanied by formation of some monoester (**9**) and dihydroxy dihydro form (**3a**) in all the media except the liver homogenate. The rate of hydrolysis of the first ester moiety is faster than the second, and a reasonable amount of monoester **9** is building up with time. The monohydroxy quaternary **10** could not be detected, except in the blood, as a very small peak that does not change significantly with time. A steady increase in the concentration of the dihydroxyquaternary **4a** was observed in all media except liver homogenate. So, it is established that this derivative, **4a**, is forming as the main product of the various interconversion routes and it is the direct precursor that should be locked in the brain in the in vivo experiment. No formation of the methoxy derivatives **7** and **8** could be detected in any of the biological fluids studied; **3a** and **4a** do not appear to be good substrates for COMT.

In Vivo Studies. The first objective of the in vivo studies was to follow the appearance and disappearance of **4a** in blood and brain following administration of **3c**. Figure 6 summarizes the results, which is very much in agreement with the concept shown in Scheme I. After one single injection of the 1,4-dihydropyridine derivative **3c** to the rate, the dihydroxy quaternary **4a** (ion), which is the only detectable derivative, could be seen to appear and then to disappear quickly from the blood, with a half-life of 27 min. On the contrary, the concentration of **4a** (ion) is increasing in the brain steadily, reaching a maximum at about 30 min following administration. The descending portion indicates a half-life of disappearance from the brain of about 3.2 h. No formation of *O*-methyl metabolites (**7** and **8**) could be detected in the brain. This confirms the in vitro results that **4a** (or **3a**) is not a good substrate for COMT.

The main remaining question was: Is dopamine finally released in the brain, at the end of this complex delivery process? Attempts to analyze for changes in brain-dop-

Table I. Comparative in Vitro Activity of 4a vs. Dopamine[a]

prolactin,[b] (ng/mg)/h							
dopamine (DA)[c]				4a[d]			
control	DA, 2×10^{-8} M	control	DA, 2×10^{-7} M	control	4a, 2×10^{-8} M	control	4a, 2×10^{-7} M
344 ± 50	355 ± 67	282 ± 34	121 ± 38*	342 ± 38	386 ± 29	250 ± 30	277 ± 32

[a] On freshly obtained anterior pituitary (AP) at 37 °C. All values are average of nine separate AP-S. * = $p < 0.05$. [b] Prolactin release rate of the incubated AP-S. [c] Weight of the AP-S: control, 4.6 ± 0.2 mg; DA treated, 4.5 ± 0.3 mg. [d] Weight of the AP-S: control, 4.6 ± 0.3 mg; 4a treated, 4.7 ± 0.4 mg.

amine concentrations, following intrajugular administration of **3c**, lead to inconclusive results. Some rats have shown an up to threefold increase in the dopamine concentrations, others practically none. Since it is possible (and even desired) that the intrinsic brain metabolism of the dopamine does not allow significant build up of its concentration, specific pharmacological activity was investigated, by using changes in the in vivo prolactin secretion. It is known that dopamine and its agonists decrease prolactin secretion following their binding to stereospecific receptors located on lactophors in the anterior pituitary (AP) gland.[18-20] This effect is dose dependent and it can also be observed in vitro, incubating anterior pituitaries with dopamine or its agonists.[21]

In the present studies, exposure of male rats to 17β-estradiol for 2 days resulted in elevated serum prolactin levels to greater than 150 ng/mL. Intravenous administration of **3c** caused a 79% decrease in serum prolactin concentrations, and this dramatic reduction was maintained through 120 min after treatment. In contrast, **4a** had no significant effect on the serum prolactin concentrations by 15 min and caused a 67% reduction by 30 min. Thereafter, serum prolactin levels increased progressively to levels that are not significantly different from vehicle-injected controls, by 60 and 120 min. These results[22] are summarized in Figure 7. The rapid onset and prolonged inhibitory effects of **3c** on prolactin secretion is consistent with the time course of the appearance of **4a** in the brain following administration of **3c**. The trapping of **4a** in the brain subsequent to iv injection of **3c** appears to provide a constant source of a potent dopaminergic agent, either dopamine or **4a** itself. The significantly lower effect of **4a** when administered iv does not clarify the question of which is the active species. This was resolved by in vitro comparison of the relative activities of dopamine vs. **4a**.

Fresh anterior pituitaries obtained from female rats were incubated with various concentrations of dopamine (DA) and **4a**, respectively, and their effects on the rate of release of prolactin were measured. It was found that at 2×10^{-8} M concentrations, neither DA nor **4a** had any effect; however, at a 2×10^{-7} M concentration, DA caused a 57% reduction of the prolactin rate secretion, while **4a** had no effect. These results are summarized in Table I.

These results indicate that if **4a** has any activity it must be significantly less than that of DA. Based on the delayed onset of the activity when **4a** was administered iv and considering the in vitro results, the most likely explanation for the high and prolonged activity of the **4a** locked in the brain following administration of **3c** is that **4a** is releasing

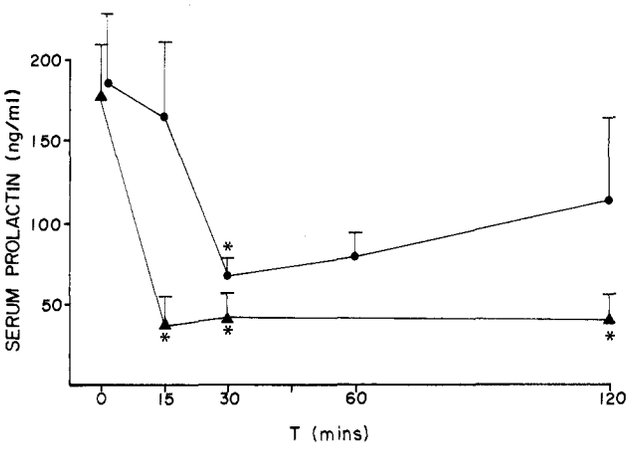


Figure 7. Effects of compounds **3c** (▲) and **4a** (●), administered iv at a 1 mg/kg dose level, on the serum prolactin levels in rats. Error bars represent SE of mean, and the asterisks represent a probability level of <0.05.

the active DA slowly in the brain.

Thus, the redox-type brain-specific dopamine delivery system can be summarized as follows: (1) use of a lipophilic dihydropyridine carrier type chemical delivery system ("pro-pro-drug" or "pro-pro-pro-drug" in the case of the catechol ester) of dopamine, which would penetrate the BBB by a passive transport; (2) a rapid oxidation in the brain of the carrier portion to the corresponding quaternary pyridinium salt, resulting in an activated amide of dopamine; the oxidation process is much faster than amide cleavage of the initial compound **3** or of **4**; (3) the ionic nature of the activated quaternary salt resulted in a significant slowdown of the efflux of this specific form through the BBB, resulting in a selective concentration enhancement of the precursor **4a** in the brain; (4) the dopamine was released from this activated form upon hydrolytic cleavage, resulting in brain-specific pharmacological activities; (5) facile excretion of the carrier portion from the brain is assured, as suggested by our earlier studies.[16,23] Thus, by this chemical-delivery system, a sustained delivery of dopamine specifically and preferentially to the brain was achieved.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are uncorrected. Elemental analysis was performed at Atlantic Microlabs, Inc., Atlanta, GA. Infrared spectra were determined with a Beckman Acculab 1 double-beam recording spectrophotometer. NMR spectra were determined by means of a Varian T60A or FX100 spectrometer. All chemical shifts reported are in δ units (parts per million) relative to Me_4Si. Ultraviolet absorbance spectra were determined by using a Cary Model 219 spectrophotometer.

***N*-Nicotinoyldopamine (5).** To a pyridine solution containing 11.7 g (0.05 mol) of dopamine hydrobromide and 6.15 g (0.05 mol) of nicotinic acid at 0 °C was added 10.3 g (0.05 mol) of dicyclohexylcarbodiimide (DCC). The reaction mixture was stirred at

(18) G. P. Mueller, J. W. Simpkins, J. Meites, and K. E. Moore, *Neuroendocrinology*, **20**, 121 (1976).
(19) W. Wuttke, E. Cassell, and J. Meites, *Endocrinology*, **88**, 737 (1971).
(20) J. A. Clemens, E. B. Smalstig, and C. J. Shaar, *Acta Endocrinol.*, **79**, 230 (1975).
(21) R. M. MacLeod in "Frontiers in Neuroendocrinology", L. Martini and W. F. Ganong, Eds., Raven Press, New York, 1976.
(22) N. Bodor and J. W. Simpkins, *Science*, in press.
(23) N. Bodor, R. G. Roller, and S. H. Selk, *J. Pharm. Sci.*, **68**, 685 (1978).

room temperature for 24 h, and the formed dicyclohexylurea was removed by filtration. The pyridine was removed in vacuo, and the residue was crystallized from water at 0 °C. The product was isolated by filtration and dried over phosphorous pentoxide. Recrystallization from 2-propanol gave 9.0 g (0.035 mol) of 70% *N*-nicotinoyldopamine, mp 159–162 °C; an aqueous solution of the compound gives a green color with Fe^{3+} and reduces $AgNO_3$: IR (KBr) 3300, 2960, 1725, 1630, 1590, 1520, 1430, 1290, 1190, 1115, 720, 710 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 9.25–6.25 (m, 7 H), 3.3 (m, 2 H), 2.65 (m, 2 H). Anal. ($C_{14}H_{14}N_2O_3$) C, H, N.

3-[*N*-[β-[3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-pyridine (6c). To a suspension of 5.16 g (0.02 mol) of finely powdered nicotinoyldopamine in 100 mL of chloroform was added 7.23 g (0.06 mol) of trimethylacetyl chloride while stirring. The mixture was refluxed for 6 h and then filtered. The filtrate was washed with water free of chloride ions, and then washed once with a 5% solution of $NaHCO_3$ and then with water. The chloroform was evaporated, and the residue was chromatographed by using a silica gel G column and 2% methanol in chloroform as the eluent. The first fraction was collected and evaporated, and the residue was crystallized from ether/petroleum ether: yield 6.2 g (73%) of a white crystalline solid; mp 112–114 °C; ^1H NMR ($CDCl_3$) δ 9.06 (brs, 1 H, C_2 pyridine proton), 8.73 (br d, 1 H, C_6 pyridine proton), 8.30–8.13 (m, 1 H, C_4 pyridine proton), 7.46–7.10 (m, 5 H, C_6H_3 + C_5 pyridine proton + CONH), 3.66 (q, 2 H, J = 6.25 Hz, N-CH_2), 3.0 (t, 2 H, J = 6 Hz, CH_2), 1.41 [s, 18 H, 2 $C(CH_3)_3$]. Anal. ($C_{24}H_{30}N_2O_5$) C, H, N.

1-Methyl-3-[*N*-[β-(3,4-dihydroxyphenyl)ethyl]carbamoyl]pyridinium Iodide (4a). To a solution of 1.26 g (5 mmol) of nicotinoyldopamine (**5**) in 10 mL of acetone was added 1.41 g (10 mmol) of methyl iodide, and the mixture was refluxed while stirring for 6 h. The acetone was removed, and the residue was crystallized from methanol/ether: yield 1.7 g (87%); mp 155–157 °C dec. An aqueous solution gives a green color with Fe^{3+}: ^1H NMR (D_2O) δ 9.30–8.28 (ms, 4 H, $C_5H_4^+$), 7.00 (br s, 3 H, C_6H_3), 4.60 (s, 3 H, N^+-CH_3), 3.80 (t, 2 H, J = 7 Hz, N-CH_2), 2.93 (t, 2 H, J = 7 Hz, CH_2). Anal. ($C_{15}H_{17}IN_2O_3 \cdot H_2O$) C, H, N.

1-Methyl-3-[*N*-[β-(3,4-diacetoxyphenyl)ethyl]carbamoyl]pyridinium Iodide (4b). To a solution of 1.71 g (5 mmol) of 3-[*N*-[β-(3,4-diacetoxyphenyl)ethyl]carbamoyl]pyridine (prepared as compound **6c**) was added 1.41 g (10 mmol) of methyl iodide, and the mixture was refluxed overnight while stirring. The acetone solution was then decanted from the insoluble oily residue. Ether was added to the acetone solution, and the solid that separated was crystallized from acetone/ether: yield 1.9 g (78%) of yellow crystalline needles; mp 171–173 °C; UV (methanol) max 215, 265 nm; ^1H NMR (D_2O) δ 8.86–7.63 (ms, 4 H, $C_5H_4N^+$), 6.66 (br s, 3 H, C_6H_3), 4.4 (s, 3 H, N^+-CH_3), 3.50 (t, 2 H, N-CH_2), 3.03 (t, 2 H, CH_2), 2.21 (br s, 6 H, 2 $COCH_3$). Anal. ($C_{19}H_{21}IN_2O_5$) C, H, N.

1-Methyl-3-[*N*-[β-[3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]pyridinium Iodide (4c). To a solution of 5.0 g (11.7 mmol) of compound **6c** in 20 mL of acetone was added 3.3 g (23.4 mmol) of methyl iodide, and the mixture was refluxed while stirring for 6 h and then cooled. An orange crystalline solid separated, which was filtered, washed with ether, and crystallized for acetone/ether: yield 5.6 g (85%); mp 163–165 °C; UV (buffer pH 7.4) max 270, 215 nm; ^1H NMR (Me_2SO-d_6) δ 7.68–7.06 (ms, 7 H, $C_5N_4N^+$ + C_6H_3 + NH), 4.56 (s, 3 H, N^+-CH_3), 3.42 (q, 2 H, J = 7 Hz, N-CH_2), 3.19 (t, 2 H, J = 7 Hz, CH_2), 1.32 [s, 18 H, 2 $C(CH_3)_3$]. Anal. ($C_{25}H_{33}In_2O_5$) C, H, N.

1-Methyl-3-[*N*-[β-(4-hydroxy-3-methoxyphenyl)ethyl]carbamoyl]pyridinium Iodide (7). *N*-Nicotinoyl-3-methoxytyramine was prepared by following the procedure used for the preparation of compound **5**. The isolated crude amide was quaternized directly with methyl iodide following the method used for the preparation of compound **4a**. Crystallization from methanol gave a yellow crystalline compound, mp 192–194 °C with an overall yield of 84%, calculated on the basis of 3-methoxytyramine starting material. The NMR (D_2O) is closely similar to that of **4a**, except for a singlet at δ 3.66 for OCH_3. Analysis ($C_{16}H_{19}IN_2O_3$) C, H, N.

1-Methyl-3-[*N*-[β-(3,4-dihydroxyphenyl)ethyl]carbamoyl]-1,4-dihydropyridine (3a). To an ice-cold solution of 1.0 g (2.5 mmol) of compound **4a** in 200 mL of deaerated water was added 1.26 g (15 mmol) of sodium bicarbonate. Nitrogen was bubbled into the mixture, and 1.74 g (10 mmol) of sodium dithionite was added gradually to the mixture while stirring. Stirring was continued for 1 h, and the mixture was then extracted twice with 50 mL of ether. The ether extract was washed with water, dried with anhydrous Na_2SO_4 and evaporated to dryness, yield 0.36 g (54%) of a yellow solid, mp 90–93 °C dec, which gives a green color with ferric chloride test and reduces alcoholic $AgNO_3$ instantly: UV (CH_3OH) max 220, 350 nm; ^1H NMR ($CDCl_3/D_2O$) δ 7.2–6.9 (ms, 4 H, C_6H_3 + C_2 dihydropyridine proton, 5.6 (m, 1 H, C_6 dihydropyridine proton), 4.6–4.4 (m, 1 H, C_5 dihydropyridine proton), 3.4 (m, 2 H, N-CH_2), 3.1–2.7 (m, 7 H, N-CH_3 + C_4 dihydropyridine protons + CH_2). Anal. ($C_{15}H_{18}N_2O_3 \cdot 0.5H_2O$) C, H, N.

1-Methyl-3-[*N*-[β-(3,4-diacetoxyphenyl)ethyl]carbamoyl]-1,4-dihydropyridine (3b). To an ice-cold solution of 1.4 g (3 mmol) of compound **4b** in 200 mL of deaerated water was added 1.5 g (18 mmol) of sodium bicarbonate. A stream of N_2 was bubbled into the mixture, and 2.1 g (12 mmol) of sodium dithionite was gradually added while stirring. Stirring was continued for 30 min, and then the mixture was extracted with ethyl acetate. The extract was washed with water, dried with anhydrous Na_2SO_4, and evaporated to dryness. The yellowish semisolid mass remaining gave a faint green color with ferric chloride test, indicating partial hydrolysis of the ester functions. It reduced alcoholic silver nitrate instantly: UV (CH_3OH) max 220, 273, 355 nm; ^1H NMR ($CDCl_3/D_2O$) δ 7.13–6.80 (ms, 4 H, C_6H_3 + C_2 dihydropyridine proton), 5.53 (d of d, 1 H, C_6 dihydropyridine proton), 4.63–4.46 (m, 1 H, C_5 dihydropyridine proton), 3.33 (t, 2 H, J = 6.5 Hz, N-CH_2), 3.06–2.66 (m, 7 H, N-CH_3 + C_4 dihydropyridine proton + CH_2), 1.8 (s, ~6 H, 2 $COCH_3$).

1-Methyl-3-[*N*-[β-[3,4-bis(pivalyloxy)phenyl]ethyl]carbamoyl]-1,4-dihydropyridine (3c). To a cold mixture of 2.0 g (3.5 mmol) of compound **4c**, 200 mL of deaerated water, and 100 mL of ethyl acetate were added 1.14 g (14 mmol) of sodium bicarbonate and 2.43 g (14 mmol) of sodium dithionite. The mixture was stirred under N_2 for 20 min. The ethyl acetate layer was separated, and the aqueous layer was re-extracted with 100 mL of ethyl acetate. The combined ethyl acetate was washed with cold deaerated water, dried over anhydrous Na_2SO_4, and distilled on rotovapor. The viscous yellow oily residue was dissolved in 5 mL of acetone, filtered under N_2 atmosphere, and then evaporated under reduced pressure. The solid residue was dried under vacuum over P_2O_5 in N_2 atmosphere. It reduces alcoholic $AgNO_3$ instantaneously and gives no color with $FeCl_3$ test: yield 1.3 g (83%); mp 45–48 °C; UV (CH_3OH) max 210, 355 nm; ^1H NMR ($CDCl_3$) δ 7.04–6.92 (m, 4 H, C_6H_3 + C_2 dihydropyridine proton), 5.71–5.61 (d of d, 1 H, C_6 dihydropyridine proton), 4.81 (brs, 1 H, CONH), 4.60–4.51 (m, 1 H, C_5 dihydropyridine proton), 3.53 (q, 2 H, J = 6.3 Hz, N-CH_2), 2.36 (br s, 2 H, C_4 dihydropyridine proton), 2.91 (s, 3 H, N-CH_3), 2.79 (t, 2 H, J = 6.3 Hz, CH_2), 1.33 [s, 18 H, 2 $COC(CH_3)_3$]. Anal. ($C_{25}H_{34}N_2O_5 \cdot 1.5H_2O$): C, H, N.

1-Methyl-3-[*N*-[β-(4-hydroxy-3-methoxyphenyl)ethyl]-carbamoyl]-1,4-dihydropyridine (8). This compound was prepared following the same method as for the preparation of compound **3c**. The crude solid obtained showed the same NMR ($CDCl_3/D_2O$) pattern as compound **3a**, except for a peak at δ 3.5 for the OCH_3 protons. It was sufficiently pure for the determination of its retention time following the HPLC method of analysis used. No trials were made for its further crystallization or elemental analysis.

Analytical Methods. A high-pressure liquid chromatography (HPLC) method was developed for the studies of the degradation of the dihydropyridine derivative. The chromatographic analysis was performed on a component system consisting of a Waters Associate Model 6000A solvent delivery system, Model U6K injector, and Model 440 dual-channel absorbance detector operated at 254 and 280 nm. A 30 cm × 3.9 mm (internal diameter) reverse-phase μBondapak C_{18} column (Waters Associates), operated at ambient temperature, 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 0.005 M solution of 1-heptanesulfonic acid sodium salt (PIC B-7 Eastman Kodak) in CH_3CN/0.01 M aqueous dibasic ammonium phosphate (2.5:1). At a flow rate of 2.0 mL/min, 4 had a retention time of 5.1 min; **6**, 11.8 min; **7**, 1.7 min; and **9**, 3.1 min. A peak was always shown at a retention time of 2.2 min,

which is thought to be a monodeacylated dihydropyridine derivative, since it eventually did result in **4a**.

Determination of the Enzymatic Hydrolytic Cleavage and Rate of Oxidation of Compound 3c. In Human Plasma. The freshly collected plasma used was obtained at the Civitan Regional Blood Center, Inc. (Gainesville, FL) and contained about 80% plasma diluted with anticoagulant citrate phosphate dextrose solution U.S.P. The plasma was stored in a refrigerator and used the next day. One-hundred microliters of a freshly prepared 0.61 M solution of compound **3c** in methanol was added to 20 mL of plasma, previously equilibrated to 37 °C in a water bath and mixed thoroughly to result in an initial concentration of 3.05×10^{-3} mol/L. One milliliter samples of plasma were withdrawn 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 Whatman 1 filter paper and analyzed by HPLC.

In Human Blood. The freshly collected heparinized blood was obtained at the Civitan Regional Blood Center, Inc. (Gainesville, FL). The blood was stored in a refrigerator and used the next day. One-hundred microliters of a freshly prepared 0.19 solution of compound **3c** in methanol 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 9×10^{-4} mol/L. One milliliter samples of blood were withdrawn from the test medium every 5 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 by using Whatman 1 filter paper and analyzed by HPLC.

In Rat Brain Homogenate. The brain homogenate was prepared by the following method. Five Sprague–Dawley rats were killed by decapitation, and the brains were removed, weighed (total weight 9.85 g), and homogenized in 49.3 mL of aqueous 0.11 M phosphate buffer, pH 7.4. The homogenate was centrifuged, and the supernatant was used for the test. One-hundred microliters of a 0.18 M solution of compound **3c** was mixed with 10 mL of homogenate, previously equilibrated to 37 °C in a water bath, to result in an initial concentration of 1.8×10^{-3} 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, and placed in a freezer. When all samples had been collected, they were centrifuged. Each supernatant was filtered through two Whatman 1 filter papers and analyzed by HPLC.

In Rat Liver Homogenate. The liver homogenate was prepared by the following method. Three Sprague–Dawley rats were killed by decapitation, and the livers were removed, weighed, and homogenized in a tissue homogenizer in 0.11 M aqueous phosphate buffer, pH 7.4, to make 20% liver homogenate. The homogenate was centrifuged, and the supernatant was used for the test. One-hundred microliters of a 0.1 M solution of compound **3c** in methanol was mixed with 20 mL of the homogenate, previously equilibrated to 37 °C in a water bath, to result in an initial concentration of 9×10^{-4} mol/L. Samples of 1.0 mL were withdrawn every 5 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 each supernatant was filtered through Whatman 1 filter paper and analyzed by HPLC.

Determination of the Concentration of Compound 4a in Brain and Blood after Parenteral Administration of 3c. Male Sprague–Dawley rats, average weight 150 ± 10 g, were used. The rats were anesthetized with an im injection of Inovar, and the jugular was exposed. Compound **3c** was injected intrajugularly in the form of 10% solution in Me_2SO at a dose of 50 mg/kg (equivalent to 64.2 mg of compound **4a**). The injection was given at a rate of 24 μL/min by using a calibrated infusion pump. After appropriate time periods, 1 mL of blood was withdrawn from the heart and dropped immediately into a tared tube containing 3 mL of acetonitrile, which was afterwards weighed to determine the weight of the blood taken. The animal was then perfused with 20 mL of saline solution and decapitated, and the brain was removed. The weighed brain was homogenized with 0.5 mL of distilled water, 3 mL of acetonitrile was added the mixture was rehomogenized thoroughly, centrifuged, and filtered, and the filtrate was then analyzed for compound **4a** by using the HPLC method. The tubes containing the blood were shaken vigorously, centrifuged, decanted, and also analyzed for compound **4a** by using the HPLC method. Quantitation was done by using a recovery standard curve obtained by introducing a known amount of **4a** in either brain homogenate or blood and then treated in the same manner.

Pharmacological Studies. In Vivo Effect on Pituitary Prolactin Secretion. Adult male rats (Charles Rivers, CD-1), weighing 200–225 g, were provided food and water ad libitum for at least 1 week prior to experimentation. In order to elevate serum prolactin levels, each rat received a single sc implant of a Silastic tube (1.57-mm interior diameter, 5 mm × 3.15 mm overall size) packed with crystalline 17β-estradiol. Two days later, the rats were lightly anesthetized with ether and a small incision was made over the right jugular vein for intravenous (iv) administration of the test drugs. Compound **4a** was injected at a dose of 1 mg/kg body weight/mL of saline, and groups of six rats were decapitated at 15, 30, 60, and 120 min later to collect blood samples. Control rats (time 0) received an iv injection of the saline vehicle and were decapitated 30 min later. Compound **3c** was dissolved in 10% ethanol in saline and was injected iv. Rats were decapitated at 15, 30, and 120 min later. Control (time 0) animals received the 10% ethanol vehicle and were sampled 30 min later.

Trunk blood was collected and allowed to clot for 2 h, and the serum was separated and stored at −20 °C for subsequent assay for prolactin concentrations. Each serum sample was assayed in duplicate by the double-antibody radioimmunoassay procedure described by the National Pituitary Agency Hormone Distribution Program. Serum prolactin concentrations are expressed in terms of the PRL-RP-2 reference preparation provided. The intraassay coefficient of variation for 10 replicate samples of pooled serum obtained from male rats was 13.8%.

The effects of compounds **3c** and **4a** on serum prolactin concentrations were evaluated by one-way analysis of variance and Student–Newman Keuls tests. A probability level of less than 0.05 was chosen for significance.

In Vitro Evaluation of the Prolactin Inhibitory Effect of 4a. Adult female rats (Charles Rivers Labs), weighing 225–250 g, were maintained on food and water ad libitum. Animals were sacrificed by decapitation; their pituitary glands were quickly removed from the cranium. The anterior pituitary (AP) of each animal was dissected into two equal halves and placed into incubation media. (Gibco's minimal essential media supplied by Grand Island Biological Co. was used.) The incubation was conducted at 37 °C, under continuous aeration (95% O_2/5% CO_2); the pH was 7.2. After 1 h of preincubation, the media were discarded and replaced with fresh ones containing either DA (2×10^{-8} M), **4a** (2×10^{-8}), or ascorbic acid (10^{-4} M). In all cases, one-half of AP received the test drug; the other, the ascorbate control. After 1 h, samples were taken from the media, and the remaining media were discarded. Fresh media containing DA (2×10^{-7}), **4a** (10^{-7}), and ascorbate, respectively, were then added. One hour later, the second samples were taken. After the 3-h incubation period, each half of the AP was weighed.

The samples were diluted 1:50 with phosphate-buffered saline and then assayed in triplicate by the radioimmunoassay method described. The data are given as nanograms of prolactin released per milligram of wet weight per hour. Paired Student's t test was used to evaluate the significance of the inhibitory effects of the test drugs on prolactin secretion. The control AP half and the drug-treated half were employed in each paired comparison.

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