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BLOOD-BRAIN PENETRATION OF 5-HYDROXYTRYPTAMINE DERIVATIVES

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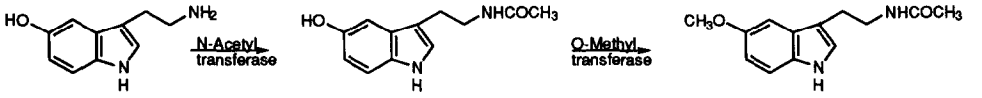
Abstract: *Glycosyl phosphate derivatives of N-acetyl-5-hydroxytryptamine were synthesized from 2-deoxy-D-glucose, D-glucose and D-mannose. These brain transport vectors for the precursor of melatonin were tested for locomotion on mice : with intracerebral and peripheral administrations, the prodrugs exhibited an inhibitory effect, whereas the same effect was observed for melatonin or its precursor only with an iv injection.*

The blood brain barrier (BBB) protects the brain against toxic effects of compounds produced at the periphery and also constitutes a serious limitation in the use of drugs directed towards cerebral targets. Among the various strategies proposed to improve the crossing of BBB by prodrugs (1-3), the use of glycosyl phosphotriester derivatives has been previously proposed (4) and we have recently shown that, in mice, the AZT delivery within the brain was markedly improved by using an oral dose of AZT glycosyl ester prodrug (5).
In the present work, we developed the glycosyl phosphotriester transport system to N-acetyl-5-hydroxytryptamine **1** which is the immediate metabolic precursor of melatonin **2**.



Serotonin — 5-Hydroxy N-acetyl-tryptamine **1** — Melatonin **2**

Melatonin is a neural hormone which exerts numerous physiological effects in brain function including behavioral changes : food and fluid intake (6), sexual behavior (7-11), sleep (12,13) and in particular locomotor activity (14-16). The clinical perspectives of this serotonin metabolite cover various fields such as aging process, cancer, epilepsy, seasonal affective disorder and circadian rhythmicity (17,18).
The herein presented experiments studied the ability of N-acetyl-5-hydroxytryptamine glycosyl phosphotriester derivatives to cross the BBB by measuring their functional cerebral effect on locomotion. This effect was compared to that of melatonin **2** itself, which exhibited a decreased locomotor activity with intracerebral injection of low doses (0.1-100 ng) (14), whereas a peripheral administration at high concentration (30 mg/kg) was shown to be inactive in mice (16).

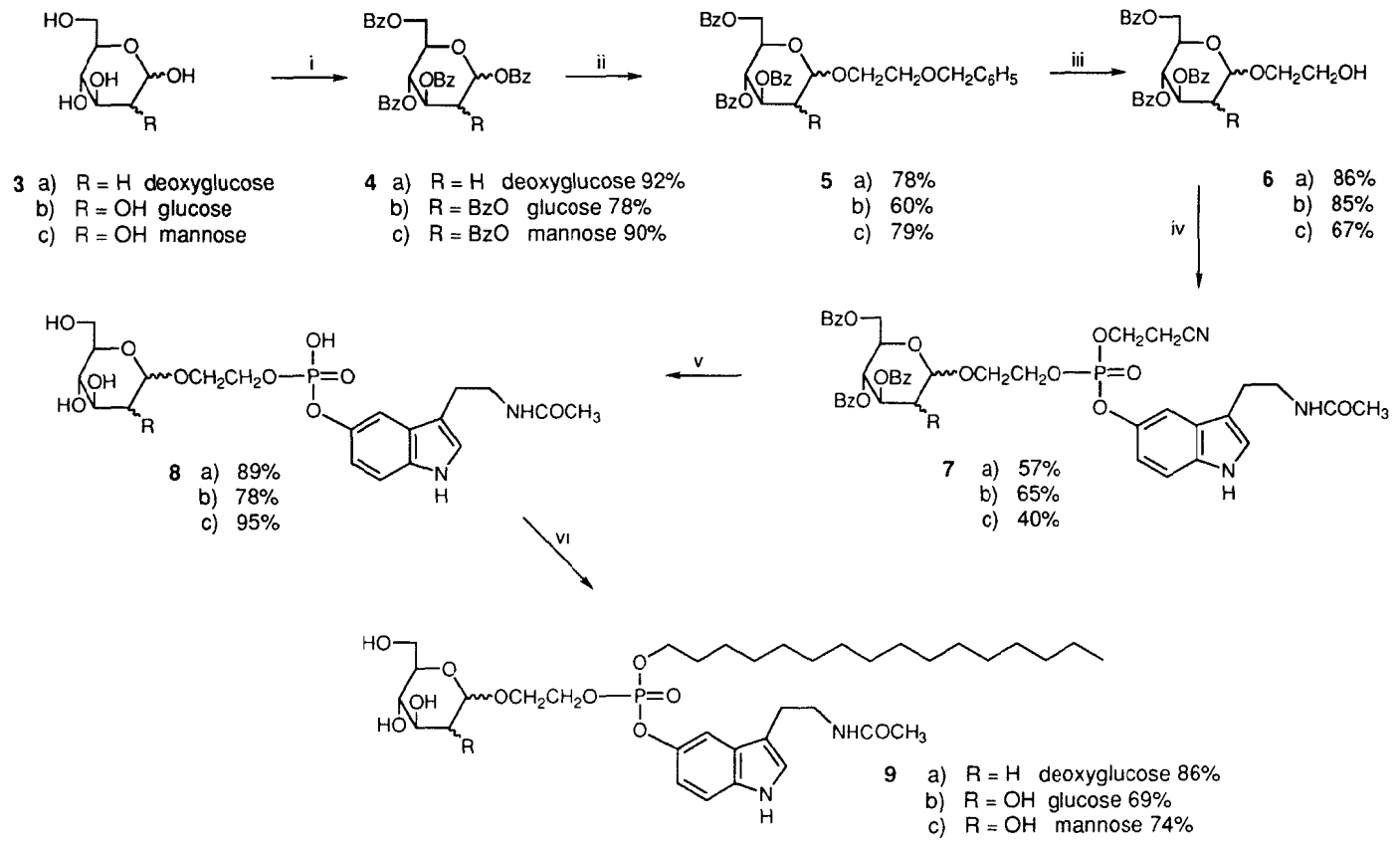


Figure 1

i) BzCl, pyridine, 0°C, 15 h. ii) a) $C_6H_5CH_2OCH_2CH_2OH$, HCl, RT 2h30; b,c) HBr, AcOH; $Hg(CN)_2$, $HgBr_2$, $C_6H_5CH_2OCH_2CH_2OH$, $C_6H_5CH_3$-CH_3NO_2, 1h30 RT. iii) H_2, Pd-C, $CH_3CO_2C_2H_5$. iv) $ClP(OCH_2CH_2CN)N(iPr)_2$, CH_3CN, $Et(iPR)_2N$, RT 15 min; tetrazole, **1**, CH_3CN, 1h RT; I_2, pyridine-THF-H_2O. v) $NaOCH_3$ 1%, 10min RT.vi) Dowex $N(Bu)_4^+$; $IC_{16}H_{33}$, CH_3CN, 80°C 15h.

Table 1. Spectral Data

^{1}H-NMR (300 MHz)	8a (D_2O)	8b (D_2O)	8c (D_2O)	9a (DMSO)	9b (DMSO)	9c (DMSO)
Sugar						
H1	4.90	4.35	4.90	4.90	4.19	4.80
H2	1.95			1.90		
H2'	1.55		3.60-3.82	1.40		
H3	3.85	4.00		3.75	3.94	
H4	3.30			3.00		
H5					2.84-3.18	3.25-3.38
H6H6'						
Sugar O-CH2-CH2-O-P	3.65	3.80	3.58	3.65	3.69	3.65
Sugar O-CH2-CH2-O-P	4.10	4.15	4.11	4.22	4.09	4.20
Tryptamine						
H2	7.20	7.20	7.20	7.20	7.21	7.20
H4	7.42	7.42	7.41	7.33	7.32	7.38
H6	7.39	7.39	7.38	7.30	7.30	7.35
H7	7.05	7.05	7.05	6.95	6.95	6.95
CH2-CH2-NH	2.90	2.92	2.91	2.76	2.76	2.78
CH2-CH2-NH	3.45	3.44	3.45	3.50	3.44	3.45
-CO-CH3	1.90	1.85	1.87	1.80	1.80	1.80
Alkyl Chain						
-(CH2)n-CH3	-	-	-	0.85	0.85	0.85
-(CH2)n-				1.23	1.23	1.20
P-O-CH2-CH2-(CH2)n-				1.60	1.59	1.60
P-O-CH2-CH2-(CH2)2-				4.08	4.24	4.07
^{31}P-NMR (121 MHz)	- 2.77	- 2.60	- 2.79	- 2.60 - 2.66	- 2.56 - 2.59	- 2.60 - 2.65
MS FAB$^+$	527.3 ($M+Na^+$)	987.4 ($M+N(BU)_4^+$)	987.5 ($M+N(BU)_4^+$)	735.7 ($M+Na^+$)	751.8 ($M+Na^+$)	751.6 ($M+Na^+$)
HPLC (min.)	11.07	7.05	7.05	15.35	13.11	13.19

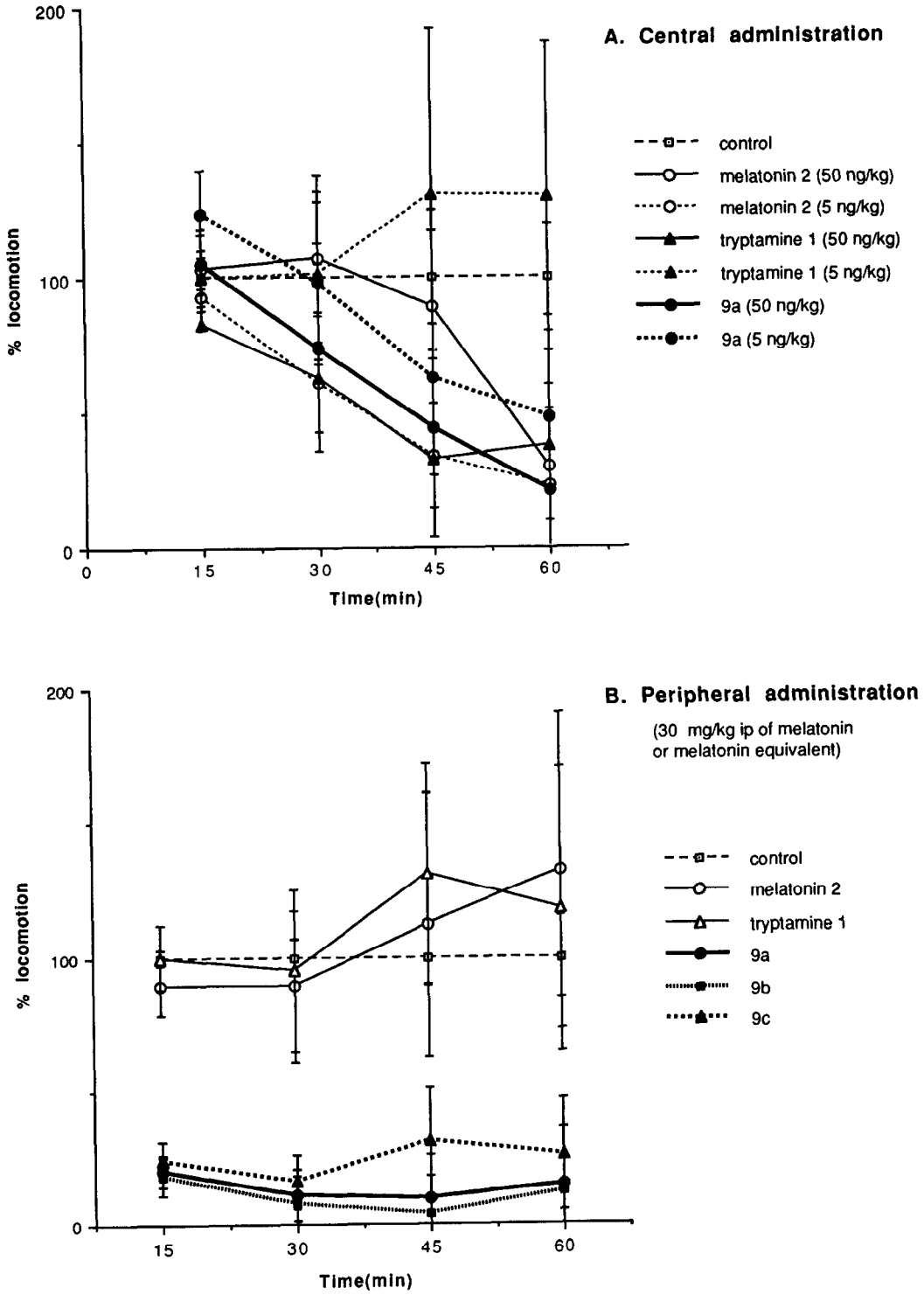
The synthetic scheme was presented on figure 1. The hydroxyethyl-D-glycopyranosides **6**, obtained in three steps for the corresponding sugars **3** (2-deoxy-D-glucose, D-glucose and D-mannose) were phosphitylated with the P^{III} reagent cyanoethyldiisopropylchlorophosphoramidite (19) ; the intermediates were activated with tetrazole, coupled to N-acetyl-5-hydroxytryptamine **1** and oxidized with I_2/THF/pyridine/H_2O in a one-pot procedure to give the indole derivatives **7** (a,b,c). These blocked phosphotriesters were purified with a column chromatography on silica gel (CH_2Cl_2-CH_3OH) and a gel filtration on LH-20 (THF-CH_3OH) before being deprotected with a 1 % sodium methylate solution into the phosphodiesters **8**. After a purification on a column of C_{18} reverse phase silica gel, the phosphodiesters were exchanged to their tetrabutyl ammonium salts and alkylated with iodohexadecane to the final phosphotriesters **9** (a,b,c) (4,19). The structure and purity of the glycosyl phosphotriesters of N-acetyl-5-hydroxytryptamine **9** were checked with NMR and mass spectroscopies, HPLC (Table 1) and combustion analysis (20).

The effects of melatonin **2**, its hydroxy precursor **1** and the phosphotriester prodrug **9a** (2-deoxyglucose series) were determined on the locomotor activity of mice with an automatized activometer. In a first experimental series, the compounds were administered by intracerebroventricular (icv) injections. Concentrations of 5 and 50 ng/kg of melatonin or the melatonin-equivalent of the phosphotriester **9a** significantly ($p < 0.05$) reduced the locomotor activity of the animals during two periods 30-45 and 45-60 min. after injection (figure 2 A). The observed effects were similar for melatonin (5 and 50 ng/kg), the prodrug **9a** (5 and 50 ng/kg) and the 5-hydroxy N-acetyl tryptamine **1** (50 ng/kg). In these assays, melatonin was more effective at 5 ng/kg than at 50 ng/kg icv ; this unexplained result was already observed (14) and might be due to the existence of stimulating properties of melatonin at high concentrations which mask the inhibitory activity exhibited at low concentrations. The fact that N-acetyl-5-hydroxytryptamine was active at 50 ng/kg strongly suggested that it was locally metabolized into melatonin which in turn exerted its inhibitory activity on locomotion. These experiments led to the important conclusion that the glycosyl phosphotriester **9a** could be metabolized in the brain tissue into N-acetyl-5-hydroxytryptamine which was further converted into melatonin, the observed physiological effects of these two indole derivatives being alike.

The peripheral administration of the prodrug **9a** at 0.01, 1, 10 (not shown) and 30 mg/kg (figure 2 B) induced a dose dependant inhibitory effect on the locomotor activity of the animals whereas parallel administrations of melatonin **2** or the precursor N-acetyl-5-hydroxytryptamine **1** at 0.01, 1, 10 (not shown) and 30 mg/kg (figure 2 B) were inactive. The prodrugs using different sugar moieties (D-glucose **9b** and D-mannose **9c**) exhibited similar activities as those observed with the deoxyglucose triester derivative **9a** (figure 2 B). Moreover, under the same experimental conditions, the glycopyranosides and hexadecanol, constituents of the active prodrugs, were devoid of activity, strongly suggesting that they were not involved in the intrinsic activity of the latter compounds. Therefore, these results indicate that the phosphotriester prodrugs of N-acetyl-5-hydroxytryptamine cross the BBB and likely are metabolized into melatonin which will act centrally. HPLC assays were carried out to determine the presence of the phosphotriester prodrugs **9** in brain tissue after ip administration : the fact that they could not be evidenced strongly suggests that the prodrugs were rapidly hydrolyzed into molecules identical to endogenous metabolites already present in the brain (22) ; however the final demonstration of this hypothesis should await for the result of labelling studies.

In conclusion, it is shown that the ability of molecules to cross the BBB could be markedly increased by using the glycosyl phosphotriester vector ; this prodrug allows the transport and the functional activity of the derived molecule and therefore may have important therapeutic interest.

Figure 2


A. Central administration
control
melatonin 2 (50 ng/kg)
melatonin 2 (5 ng/kg)
tryptamine 1 (50 ng/kg)
tryptamine 1 (5 ng/kg)
9a (50 ng/kg)
9a (5 ng/kg)
% locomotion
200
100
0
0
15
30
45
60
Time(min)
B. Peripheral administration
(30 mg/kg ip of melatonin
or melatonin equivalent)
control
melatonin 2
tryptamine 1
9a
9b
9c
% locomotion
200
100
0
15
30
45
60
Time(min)

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20. Correct elemental analysis (C,H,N,) were obtained for the final compounds. Mass spectra were performed with a VG70-250 instrument. Analytical HPLC was performed on a Hewlett-Packard 1090M series and a Nucleosil 5-C18 (5 μm) column (15 x 046 cm) with a 20 min. gradient (1 ml/min.) of 0-25 % acetonitrile in 0.01 M triethylammonium acetate (pH 7.0) for phosphodiesters **8** and 50-95 % acetonitrile for phosphotriesters **9**. ^{1}H and ^{31}P NMR spectroscopies were recorded on a Bruker AC-300 spectrometer and referenced to internal tetramethylsilane.
21. Male Swiss mice (IFFA CREDO, France) weighting 28 to 32 g were maintained at constant temperature (22°C) and a light/dark cycle 12/12 with food and water *ad libitum* for a least one week before experimentation.
    The animals received intraperitonealy vehicle (distilled water 0.5 ml per 20 g of body weight) or the drug at the indicated concentration (n=16 at least in each group). The values expressed in mg/kg refer to melatonin itself or the melatonin-equivalent of the derivatives **9**. The cerebral administration of the drugs (or of the vehicle) consisted of an icv injection (5 μl per 20 g body weight) using a Hamilton syringe equiped with a needle (0.45/10 mm). The injection was realized at 1.5 mm to the right of the median line, 5 mm behind the orbital line and lasted for 30 seconds.
    The injected animals (n=16 at least in each group) were let to recover in their cage for 15 min. Then, they were introduced into the activometer chambers (Appelab). The horizontal locomotor activity of the animals was recorded using photocell electrical activity for 4 successive periods of 15 min. each.
22. In three independant experiments, mice received vehicle (control) or the prodrug **9a** (30 mg/kg ip) and were sacrificed one hour later. The brains of the animals were dissected out and homogenized in Tris HCl (5 m M) buffer containing sucrose (0.32 M) at pH 7.4. The homogenate was centrifuged (1000 g for 5 min.) to obtain a crude mitochondrial fraction, collected and centrifuged (12000 g for 30 min.). This preparation containing synaptosomes was extracted with acetonitrile-water (3/1 v/v) at 22° C. The extract was analyzed by HPLC as described in (5). Under these experimental conditions, the prodrug **9a** was not found in brain extracts, whereas when it was added directly into the homogenate and incubated at 37° C for 15, 30 or 60 min. it was actually detected in the extract (~ 18 % recovery). The sensibility of the detection was 0.01 nmole.

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