A NUCLEAR MAGNETIC RESONANCE STUDY OF THE INTERACTION OF SEROTONIN WITH GANGLIOSIDES*

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1. Introduction

Serotonin has important central nervous system functions [1]. It has been implicated as an excitatory neurotransmitter and its altered levels contribute to psychopathic conditions like schizophrenia [2]. The high specificity of neurotransmitters suggests that their actions are mediated by specific binding to a receptor site on the synaptic plasma membrane. Marchbanks has shown that serotonin binds to nerve ending particles with three major binding affinities [3]. The high affinity binding site ($K_a \sim 10^6$ M^{-1}) is recovered in *n*-butanol extracts of brain. Binding is inhibited by d-LSD and destroyed on neuraminidase treatment. Gangliosides have been suggested as the serotonin binding component and consequently as the possible 'receptor' site. Gangliosides are generally located on the outer surface of nerve cells [4]. The ability of purified gangliosides to bind serotonin in aqueous solution has been demonstrated [5,6]. We describe in this communication the results of an ^1H n.m.r. study, of the interaction of serotonin with monkey brain gangliosides.

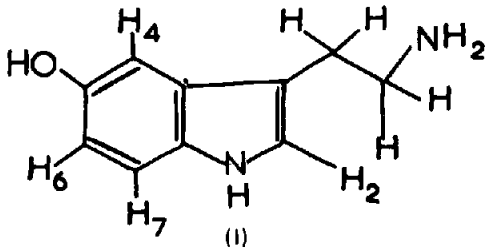
2. Experimental procedures

Mixed gangliosides were isolated from monkey brain, by the procedure described by Gammack [7]. Concentrated aqueous solutions of gangliosides were exhaustively dialysed against 0.1 M EDTA, glass distilled water and freeze dried, to yield a cream coloured fluffy solid. Brain lipids were extracted by the method of Bligh and Dyer [8]. Dipalmitoyl lecithin and serotonin were obtained from Sigma Chemical Co.

N.m.r. spectra were recorded at 60 MHz, on a Varian T-60 spectrometer at 35°C. Serotonin, as the oxalate or creatinine sulfate salt, was dissolved in D_2O (30–50 mg/ml) and solutions containing weighed amounts of gangliosides and serotonin were added, to vary the glycolipid concentration. pH of n.m.r. samples were maintained at ~4.0. Increasing the pH to 6.0 had no effect on the studies reported. Fluorescence spectra were recorded on a Perkin-Elmer MP203 spectrofluorimeter.

3. Results and discussion

The n.m.r. spectrum of the indole protons of serotonin (I) is shown in fig.1a and the peak assignments are indicated.



(I)

Fig.1 b–d, show the effects of increasing concentrations of brain gangliosides on the serotonin spectrum. There is a dramatic broadening of the aromatic resonances, without observable shifts at 60 MHz.

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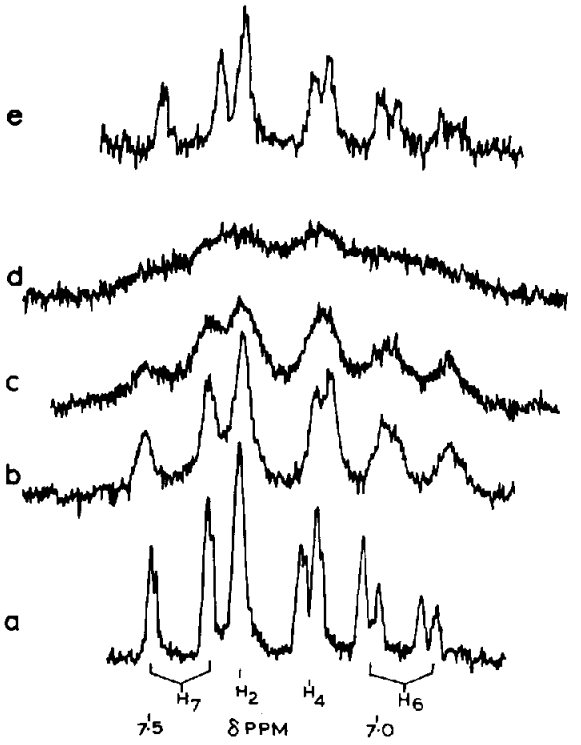

Fig.1. Aromatic proton resonances of serotonin as a function of ganglioside concentration. Serotonin concentration 40 mg/ml. Ganglioside concentration (a) 0, (b) 5.6 mg/ml, (c) 15.8 mg/ml, (d) 47.5 mg/ml and (e) serotonin (33 mg/ml) + lecithin (36.5 mg/ml).

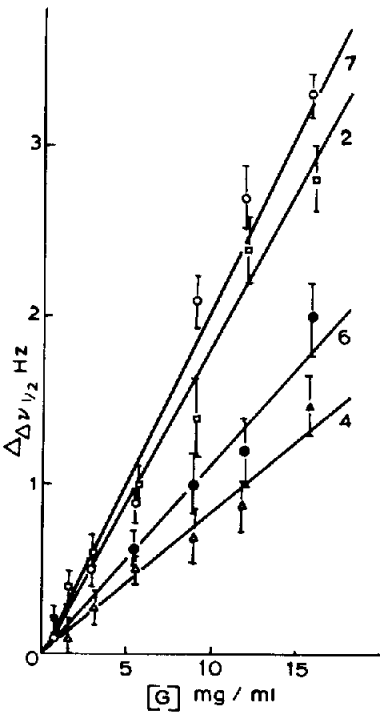

Fig.2. Plot of the line broadening ($\Delta_{\Delta\nu_{1/2}}$) of the aromatic resonances of serotonin as a function of ganglioside concentration.

This is clear evidence that exchange, of serotonin molecules between bound and free states, is fast on the n.m.r. timescale. Gangliosides form high mol.wt. micelles (200 000 to 450 000), in aqueous solution. The critical micelle concentration for brain gangliosides is 0.015%, with about 200 molecules per micelle [7]. The n.m.r. experiments reported here, were carried out at ganglioside concentrations varying from 0.078% to 1.6%. The correlation time, for serotonin bound to micellar aggregates, is likely to be large leading to line broadening. Fig.2 is a plot of the change in line width of the indole protons as a function of ganglioside concentration. It is clearly seen that H_2 and H_7 are broadened to a greater extent than H_4 and H_6. The $-CH_2-$ protons of the sidechain also broaden. However the complex nature of the multiplet prevents measurement of individual line widths. The observation of differential line broadening for protons, at adjacent positions on an aromatic ring is evidence for the contribution of intermolecular dipolar interactions in the bound state. In free serotonin, H_6 and H_7 must relax by mutual dipole–dipole effects due to their proximity. Differential broadening implies the close approach of a proton on the ganglioside to H_7. Similarly for H_2, hydrogen atoms on the glycolipid appear to contribute to nuclear relaxation, by their closeness in space. Intramolecular contributions arising from the ethylamine sidechain cannot be ruled out, although geometric constraints make this less likely. Differential changes in correlation times for the four nuclei are ruled out, as they are attached to the rigid framework of the indole system. Intermolecular dipolar interactions have been shown to cause differential relaxation of aromatic protons, on sulfonamide inhibitors bound to carbonic anhydrase [9]. Nuclear Overhauser Effects have been used to conclusively establish the existence of intermolecular dipolar interactions, in the complex formed between

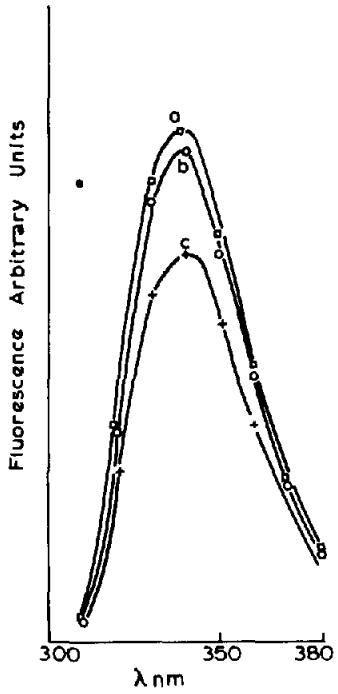

Fig.3. Uncorrected fluorescence spectrum of serotonin in the presence of gangliosides. Serotonin (10 μg/ml) in 10 mM Tris-HCl buffer, pH 7.4. Ganglioside concentration, (a) 0, (b) 20 μg/ml and (c) 200 μg/ml. Excitation wavelength, 310 nm.

tripeptides and neurophysin-II [10]. These experiments allow elucidation of the nature of the amino acid residues at the protein binding site. In principle, the identity of the spatially proximate hydrogen atoms in the ganglioside–serotonin complex can be determined by this technique.

Fig.1e shows that addition of dipalmitoyl lecithin dispersions to serotonin solutions does not cause appreciable broadening of the resonances. Whole brain lipids were also without effect. Attempts to detect line broadening on addition of subcellular fractions enriched in plasma membranes, from brain tissue, were also unsuccessful. Gangliosides are known to bind divalent ions [11]. The presence of trace paramagnetic ions like Cu^{2+} may lead to line broadening. Control experiments, carried out by addition of EDTA to n.m.r. samples and by extensive dialysis of ganglioside solutions against 0.1 M EDTA, yielded reproducible line broadening data. Further $CuCl_2$ up to a concentration of 0.35 mM did not affect the serotonin spectrum. These results suggest a specific and strong interaction of serotonin with gangliosides, that is not mimicked by other lipid dispersions. Fig.3 shows the quenching of serotonin fluorescence on addition of gangliosides. This is also suggestive of an intermolecular interaction. Preliminary n.m.r. experiments indicate that the phenothiazine drug chlorpromazine, which has antiserotonergic activity [12], also binds to brain gangliosides. In view of the postulated role for gangliosides as the 'serotonin receptor' and the importance of phenothiazine drugs in medical practice, further spectroscopic investigation of this binding process is in progress.

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