Binding of Tryptamine and Allied Compounds to Nucleic Acids

In discussions of the nature of the receptor site for central transmitters in neuronal membrane it is usually assumed that it must be composed of protein or perhaps glycoprotein. Because RNA has been reported to be present in membrane[1–5] and because it may well have functions in the cell besides its direct involvement in protein synthesis, however, it is possible that ribonucleoprotein may have a part to play in membrane function (for example, opening ionic channels, and making up part of the receptor site in neurones). A detailed development of this hypothesis has been presented elsewhere[6,7]. This article is concerned with experiments attempting to determine the mechanism of binding of tryptamine and related compounds to nucleic acids.

Siegal and Salinas[8] state that fluorescence studies reveal a strong interaction between serotonin and nucleic acids. Yielding and Sterglanz[9] report an interaction between LSD and native DNA, but not denatured DNA or RNA, using a reduction of native fluorescence and a change in ultraviolet absorption as criteria for binding. They do not specifically mention the alternative explanation of quenching of the photon absorption type, however. We have therefore repeated and extended their investigations.

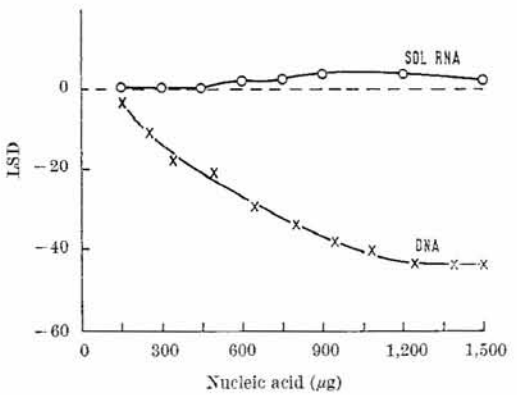

Fig. 1. The percentage decrease in the fluorescence of 10 μg [+]-LSD in 2 ml. *tris* buffer (330/430) with added increments of (×) DNA (Sigma D1501) and (○) soluble RNA (Sigma 7250).

All compounds were dissolved in *tris* buffer at *p*H 8·0 and readings were taken at room temperature with a Farrand spectrophotofluorometer against blanks for the various components of the reaction in isolation. The procedures were timed to allow the same period of reaction in each case. Fig. 1 shows the decrease in the native fluorescence of (+)-LSD when increments of DNA were added and the lack of any apparent reaction with soluble RNA. These reaction mixtures were tested 4 h later and showed no change. Fig. 2 shows the reactions between 5HT and nucleic acids indicating that it interacts equally with DNA and RNA. Nucleic acids do not absorb ultraviolet light in the range 310–450 nm. Activation of 5HT at 310 nm with a recording scan produced the same result as described here and the activation and emission frequencies for LSD lie well outside the frequencies at which nucleic acids absorb; therefore these results indicate binding rather than quenching of the photon absorption type. Addition of DNA also reduced LSD absorption as reported by Yielding and Sterglanz[9].

Tryptamine and its derivatives could bind to nucleic acids in one of four ways: (i) by ionic bonds between the positively charged protonated amine N and the phosphate groups; (ii) by intercalation between bases (RNA) or base pairs (DNA) and binding by π orbital overlap; (iii) hydrogen bonding to the spare –NH and O orbitals of the base pairs in the floor of the two grooves in the

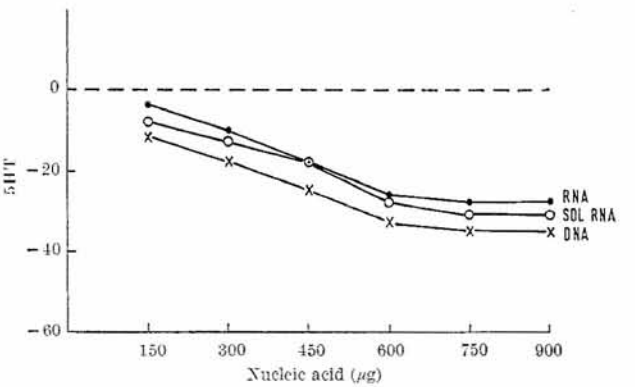

Fig. 2. As for Fig. 1 for 10 μg 5HT (290/330) (RNA is Sigma 7125).

helix; and (iv) single-stranded nucleic acids would offer a fourth way of bonding by hydrogen bonds to the unoccupied sites on the unpaired bases. In order to ascertain which obtain in this case, we carried out the following experiments: (1) If the binding is ionic in nature to phosphate, it should be modified by changes in *p*H and by blockade of the phosphate groups by divalent metallic ions such as Mg^{++} that are known to bind strongly to these groups. Fig. 3 shows that altering the *p*H in the range 4–12 had no effect on the binding of tryptamine to DNA. At *p*H 13 the native fluorescence of tryptamine was reduced by a factor of 10 (indicating instability) and binding was reduced. Binding to RNA was increased at acid *p*H (RNA is unstable above *p*H 8). Fig. 4 shows that the addition of up to 300 isomoles of $MgCl_2$ to a mixture of 9·1 μg of tryptamine and 600 μg nucleic acid had no effect on the interaction. Likewise triethanolamine (to 100 isomoles) has no effect either. These results suggest that

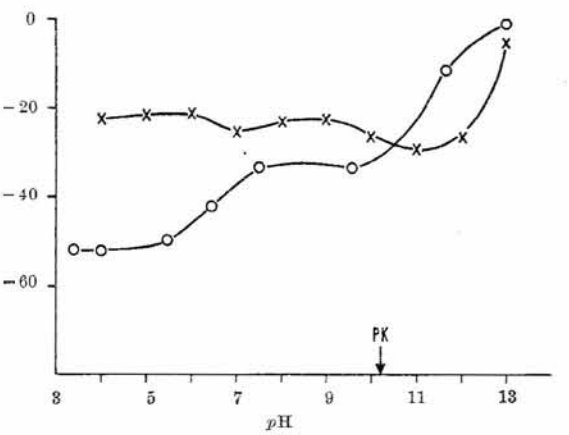

Fig. 3. The effect of *p*H on the reduction of native fluorescence of 9·1 μg of tryptamine on adding 300 μg DNA (×) and 300 μg RNA (○): *p*H altered by 0·1 N HCl or NaOH.

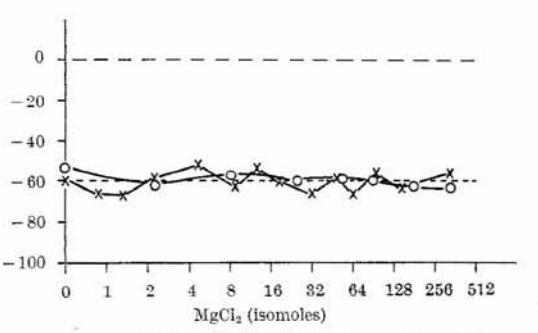

Fig. 4. The effect of adding $MgCl_2.6H_2O$ to the reaction mixture as in Fig. 3. ×, DNA, ○, RNA.

the binding of tryptamine to DNA and to RNA at physiological *p*H is not ionic but that a proportion of tryptamine may bond to RNA phosphate or base NH_2 at acid *p*H. In the case of 5HT the reaction is difficult to interpret as 5HT and $MgCl_2$ themselves interact, whereas tryptamine and $MgCl_2$ do not. We also noted that $MgCl_2$ increased the native fluorescence of RNA (by 60 per cent with 16 isomoles $MgCl_2$) at the 350/450 peak. (2) We then tested the reaction of a number of analogues of tryptamine. The results are shown in Figs. 5 and 6. In each case the amount of compound used was 150 molar with 10 μg 5HT. The interpretation of these figures in terms of the number of molecules binding per base pair is complicated by the fact that a reduction of native fluorescence could be a function of both the number of molecules bound and the amount of fluorescence lost on binding—that is, bound molecules could still fluoresce feebly. This point would need radioactive equilibrium dialysis techniques to clear up. Furthermore, the drug may be binding to only one type of RNA in the mixture of RNAs in the commercial sample. The following may, however, be noted on the basis of these data.

The compounds appear to fall into three groups. If we take tryptamine itself as a standard, equilibrium is reached at approximately 9 μg tryptamine and 1,200 μg nucleic acid. The reduction in fluorescence values is decreased by substitution in the 5 position by —OH or —OCH_3 and is increased by N,N-dialkylation with larger groups than methyl (for example, ethyl, *iso*propyl, butyl, benzyl). Of the latter compounds the di*iso*propyl and dibutyl derivatives appear to react more vigorously with RNA than with DNA. One possible explanation for these differences may derive from the fact that 5HT in solution may form a complex with itself (dimer) (by π cloud stacking and an ion-dipole bond between the N of one molecule and the Oδ− of its companion). This complex might not itself be able to bond to nucleic acid. 5HT, bufotenin and 5-O-methyl bufotenin could form such complexes and thus less would be available to bond to nucleic acid. Tryptamine would form such a complex to a lesser extent (its N could bond directly to the π cloud). In the case of the higher dialkylated products the ion-dipole bond would be lessened by steric hindrance and thus more of the compound would be available to bond to nucleic acid.

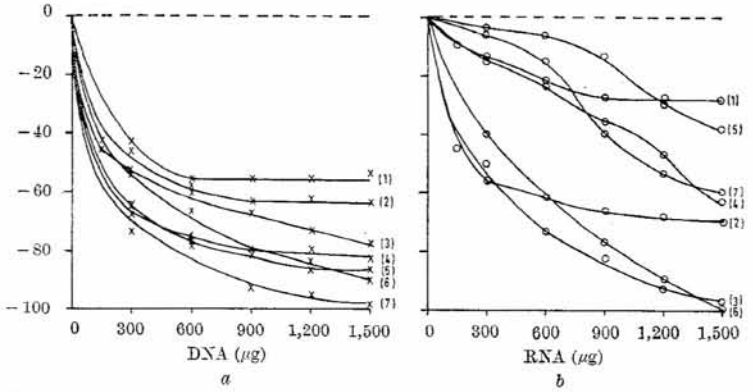

Fig. 6. Reduction in fluorescence values for further derivatives of tryptamine on interaction with (*a*) DNA; (*b*) RNA. (1) 5-benzyloxy-N,N-dimethyltryptamine; (2) N,N-dibenzyltryptamine; (3) 3(2-dehydro*iso*indolylethyl)indole; (4) 5,6,7,N,N-pentamethyltryptamine; (5) 6-chlorotryptamine; (6) 7-methyltryptamine; (7) 6-chloro-N,N,dimethyltryptamine.

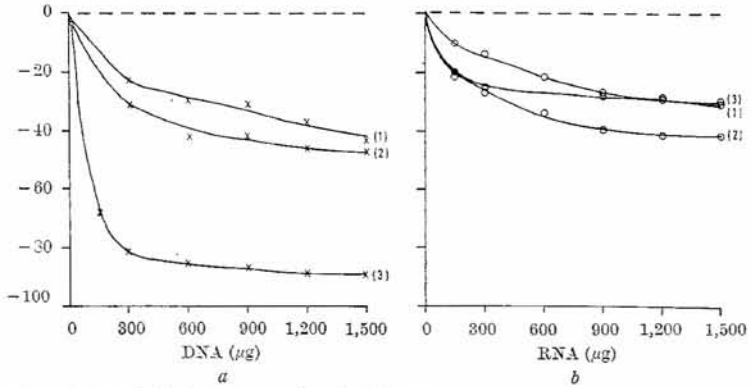

Fig. 7. Reduction in fluorescence values for (1) 1,2,3,4-tetrahydroharmine; (2) harmaline; (3) harmine interacting with (*a*) DNA; (*b*) RNA.

Moreover, a stereochemical study[6] indicates that 5HT should bind more strongly to helical RNA than to either single strand RNA or DNA.

Other substitutions on the ring induce a different reaction. Fig. 6 shows the results for seven additional compounds. This indicates that compounds with bulky ring substituents in the 5 or 6 positions (particularly 6-chlorotryptamine) have higher reduction in fluorescence values with DNA than with RNA. Thus compounds with bulky N substituents appear to bond better to RNA and some with bulky ring substituents to DNA. Fig. 7 shows that there is not much difference in the reduction in fluorescence of harmaline and tetrahydroharmine in reaction with DNA and RNA, but harmine with its bulkier ring system appears to bind better to DNA.

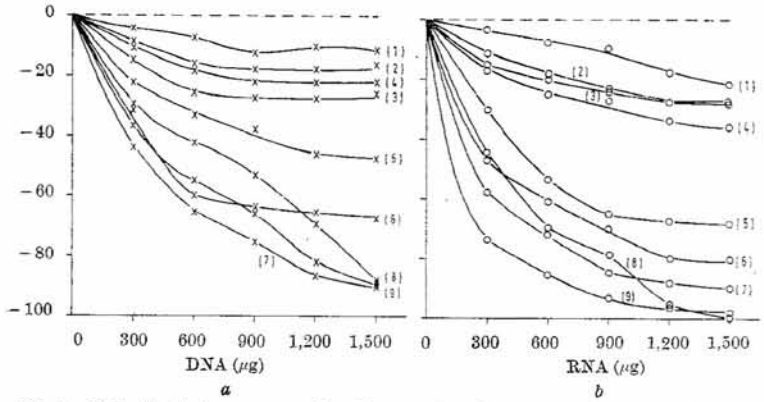

Fig. 5. Reduction in fluorescence values for a number of tryptamines on interaction with (*a*) DNA; (*b*) RNA. (1) 5-hydroxyindoleacetic acid; (2) N,N-dimethyl serotonin (bufotenin); (3) 5-hydroxytryptamine; (4) O-methyl bufotenin; (5) dimethyltryptamine; (6) tryptamine; (7) N,N-diethyltryptamine; (8) N,N-di-*iso*propyltryptamine; (9) N,N-dibutyltryptamine.

These facts may be explained as follows. (i) Tryptamines may not bind to the phosphate groups of nucleic acid because the lipophilic nature of their molecules would inhibit binding to so hydrophilic a region. (ii) If the tryptamines bind by lipophilic interactions with nucleic acids, that is by intercalation between the bases of RNA and base pairs of DNA, efficient binding will require maximum π cloud overlap. In the case of RNA this will be obtained if the tryptamine binds between two ordered purine bases with its side chain "out", in which case a bulky substituent in the 5 or 6 position will push the molecule "out" so that maximum π cloud overlap is hindered. But a large group on the side chain will not affect binding as this will lie free. In the case of helical nucleic acid, the π cloud is shared across the hydrogen

bonds linking the two bases. Thus the relatively small tryptamine molecule (as compared with a typical DNA intercalator such as proflavine) could tolerate a ring substituent such as Cl increasing its length better than it would a bulky group on the N that also increases its thickness. Likewise the bulky three and four ring molecules such as harmine and LSD would have much more π cloud overlap capacity in DNA than in single strand RNA. Thus this evidence would appear to support the hypothesis that tryptamine and its relatives bind to nucleic acids mainly by intercalation. Wagner[10] reaches this same conclusion with respect to the binding of LSD to DNA.

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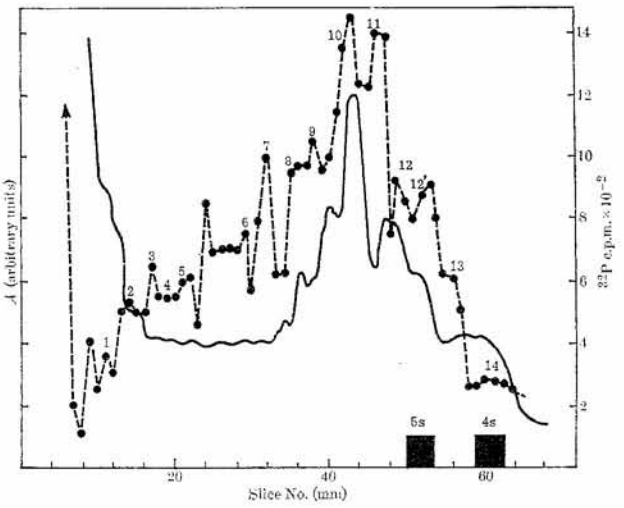

Fig. 1. Distribution of radioactive BHK-21/C13 nuclear low molecular weight RNA after electrophoresis on 7·5 per cent polyacrylamide gels. 10^8 cells were cultured for 24 h as a monolayer in an 80 ounce rotating bottle in 50 ml. phosphate-free Eagle's medium supplemented to 10 per cent with dialysed calf serum containing 500 μCi ^{32}P-orthophosphate (70 Ci/mg phosphorus). The cells were then washed with non-radioactive medium, removed by trypsin treatment, and nuclei free of cytoplasm were isolated using 'Tween 80' as described previously[6]. ^{32}P-RNA prepared from the nuclei using the hot-phenol detergent technique[6] was electrophoresed through 7·5 per cent polyacrylamide gels[6]. The gels were then sliced into 1 mm sections, dried on filter disks and assayed for radioactivity (● - - - ●). The absorbance pattern (———) is obtained as a result of electrophoresing unlabelled nuclear RNA simultaneously as described in the text. The distance migrated by cytoplasmic 5*S* and 4*S* species in similar conditions is also shown and the direction of migration in all cases is from left to right.

Synthesis of Small Nuclear Ribonucleic Acids of Mammalian Cells in relation to the Cell Cycle

Several distinct species of low molecular weight RNA from nuclei and nucleoli of mammalian cells[1–6] can be resolved by polyacrylamide gel electrophoresis. Labelling studies with HeLa cells[7] show that the low molecular weight RNA is metabolically stable and partitioned between nucleolus and nucleoplasm. We report here the timing of its synthesis in synchronized cell cultures.

Baby hamster kidney cells (BHK-21/C13) were cultured as monolayers in phosphate-free Eagle's minimal essential medium supplemented with dialysed calf serum to deplete the cellular phosphate pools before labelling the cells with ^{32}P-orthophosphate. DNA synthesis in each culture was arrested by addition of aminopterin. After 12 h the blockage imposed on DNA synthesis was released by the addition of thymidine. ^{32}P-orthophosphate was added to each culture and incubation continued for various times. Cells synchronized by this procedure[8] show a peak of maximum DNA synthesis 3 h after release from the blockage (see Fig. 2).

RNA was then extracted from the nuclei of these cells and electrophoresed through 7·5 per cent polyacrylamide gels. Because the amount of nuclear RNA present in the ^{32}P-labelled samples was small, unlabelled nuclear RNA was electrophoresed in identical conditions to act as marker. The gels were then stained with toluidine blue and scanned with a densitometer. A typical scan (Fig. 1) reveals numerous bands corresponding to low molecular weight RNA species of the nucleus. Fig. 1 also shows the resulting distribution of ^{32}P-radioactivity in a number of low molecular weight RNA moieties (designated 1 to 14) after labelling a randomly growing cell population for 24 h. Such a picture is similar to that obtained by Weinberg and Penman[7] using 10 per cent gels and the number of peaks was constant whatever labelling period greater than 1 h was used, although the maximal level of label in each peak varied. Peak 14 of Fig. 1 has a mobility comparable with that of cytoplasmic 4*S* RNA, and, as also observed by Weinberg and Penman[7], there was a double peak of radioactivity (designated 12 and 12′) with the electrophoretic mobility of 5*S* RNA. That these low molecular weight nuclear species are in fact RNA and not fragments of DNA is shown by their lability to alkali. When the labelled nuclear RNA preparations are incubated at 37° C in 0·3 N KOH before electrophoresis the maximal level of radioactivity in the region of these bands is reduced by 90–95 per cent. The excluded material (see Fig. 2*a*) is a mixture of high molecular weight RNA (18*S* and larger) and DNA. The maximal amount of radioactivity corresponding to the numbered species of low molecular weight RNA, shown in Fig. 1, was then examined at each hour after release from aminopterin block (Fig. 2 *b n*). It seems that certain of the low molecular weight nuclear RNA species are probably synthesized only at, or just after, the time of peak DNA synthesis during the S phase of the cell cycle, namely components 1–8 and 13, while other species 9, 10, 11, 12, 12′ and 14 appear to be synthesized continually. To verify that the aminopterin treatment had not interfered with the normal synthesis and maturation processes of other RNA species, such as ribosomal RNA, which has been shown to be synthesized throughout the cell cycle in cells synchronized by mitotic selection[9], cells cultured and blocked as before were labelled with ^{32}P-orthophosphate for 1 h at hourly intervals after release from aminopterin blockage and cytoplasmic RNA analysed by zonal ultracentrifugation. The amount of labelled 28*S* and 18*S* ribosomal RNA that appeared in the cytoplasm after each hourly pulse was similar up to 10 h after release from aminopterin blockage.