A Postscript

XVIII. A PROSTAGLANDIN-RNA COMPLEX AS A POTENTIAL RECEPTOR SITE:

J. R. Smythies

If RNA takes part in the molecular configuration of the 5-HT receptor site, it could possibly play a role in other receptors, and a complete theory detailing the stereochemical factors involved has been worked out (Smythies, 1969, 1970). The main points of this development may be summarized as follows:

The role of RNA in membrane may be to control the opening of ionic and amine transport channels through the membrane. There are a number of possible mechanisms here. RNA can change its configuration either by hydrogen-bond disruption converting double-stranded helical RNA to the single-stranded form or by alterations in the degree of torsion of the helix. In the former case, the ionic channel might actually run through the RNA helix. When hydrogen-bonded, the base pairs would form a neat shutter, closing the channel. If the hydrogen bonds were disrupted, the channel would open. In the latter case, the channels could be formed out of the grooves in the sides of the helix, roofed over by some molecule such as the polyamines, making two spiral chutes through the membrane, a mechanism first suggested by Melnechuk (at the Neurosciences Research Program). The aperture of these channels would be controlled by the degree of torsion of the helix. In the former mechanism again, the function of the transmitter might be to bind the RNA and initiate channel opening by disrupting the hydrogen bonds linking the bases. The second mechanism might be concerned with different transport mechanisms (e.g., for amines) as the degree of torsion of the helix is a function of the local ionic environment. A third possible mechanism would locate the channel in protein or in the ordered water-lipoprotein model postulated by Schultz (1969), and the function of RNA would be to transmit its configurational change to the protein.

Now if we regard the receptor site in neuronal and other excitable membrane as composed of a portion of RNA, it is possible to see what factors may determine whether a particular site is muscarinic, nicotinic, adrenergic, etc., because the molecular configuration of RNA is known precisely, and there are only a few permitted sources of variation that

might serve as the basis for transmitter specificity. In the case of protein, of course, there is a vast number of ways in which receptor sites could be constructed, and so any attempt to match receptors and "sites" in protein would be hopeless. But if the basic hypothesis is made instead that the primary receptor site is located in RNA and not protein, progress can be made. Of course protein may be necessary for receptor activity, but as a secondary constituent, and not as the primary binding site.

The factors that can distinguish one segment of helical RNA from another are as follows:

1. The particular base pairs involved.
2. Whether the major or minor groove is exposed at the surface.
3. The sense of the helix, i.e., whether it is wound right- or left-handed.
4. Steric hindrance or masking effects exerted by other molecules bound to the RNA.
5. The degree of torsion of the helix.

There are three possible sites for transmitters to bond to helical RNA: (1) the spare NH groups and O orbitals of the hydrogen-bonded base pairs, (2) the lipophilic intercalation site, and (3) the ionic phosphate groups. However, the latter offer no source of variability, and it is difficult to see how any transmitter binding here could effect any notable change in the RNA. However, if we consider the possible arrangement of the NH and O groups, the following may be observed. The adenine-uracil base pair would not seem adapted for any role of this kind in the minor groove, as the adenine lacks an NH_2 group, and there is no hydrogen bond at risk. Moreover, the putative binding site (to guanine NH and cytosine O) is surrounded by the highly hydrophilic and negatively charged phosphate groups that would bind many molecules such as acetylcholine, with its quaternary N^+, and that would form the focus for the formation of an ordered water shell that might encroach on the primary site. However, during the course of a systematic analysis of the stereochemical relations between membrane-active compounds and RNA, I noticed that the prostaglandins bear a striking stereochemical relation to helical RNA, shown in Figure 44. Two prostaglandin molecules (type E1 or F1α) will bond to a four base-pair sequence of RNA by four hydrogen bonds each (carbonyl O to the ribose OH on base pair 3; the 9 O (equally OH) to ribose OH on base pair 1; the 11 OH to guanine NH; the 15 OH to cytosine O on base pair 1, and any 19 OH to the ribose ring O on base

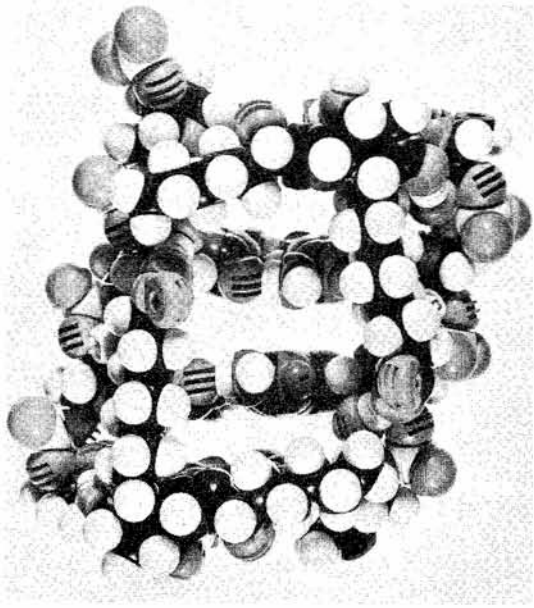

Figure 44. The prostaglandin-RNA complex. [Smythies]

pair 1 on the other side. The two prostaglandin molecules may be seen in the figure to form a lipophilic rhomboid around the postulated primary binding site, i.e., the NH and O's on base pairs 2 and 3.

It is possible to demonstrate by molecular models that muscarine (which is highly stereospecific) will bind only to a GC:GC combination in this lipophilic cup on the minor groove (Figure 45) as follows: ring O to guanine NH on base pair 2, and hydroxyl H to prostaglandin ionic O. This locates the quaternary N^+ directly over the cytosine O on base pair 3; thus it could disrupt the hydrogen bond joining this base pair. The helix must be wound right-handed. Note the degree of staggering on the base pairs owing to the helical "staircase" formation. The two "outer" sites cannot be used for bonding because they are sterically hindered by the prostaglandin. In the same way, nicotine will bind only to a CG:GC combination (both protonated N's hydrogen bond to cytosine O's; pK 6.16:10.96).* In the muscarinic site, the ACh can bind by its "ether" O to the NH on guanine on base pair 2, via its N^+ to the O on base pair 3, and via its carbonyl O to the prostaglandin carboxy OH. In the nicotinic site, ACh binds by its carbonyl O to the guanine NH on base pair 2, and by its N^+ to cytosine O on base pair 3. The "ether" O is not used for binding. The

*A more probable and sterically better fit for nicotine is obtained by bonding with the pyrrolidine N^+ and the δ^+ of pyridine.

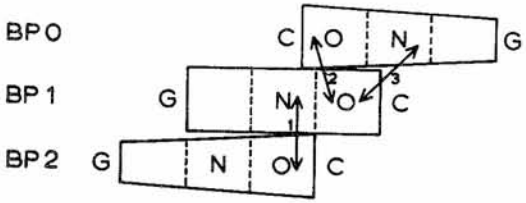

Figure 45. [BP0 + BP1] and [BP1 + BP2] should be examined as though they were two separate figures. [BP0 + BP1] illustrates the "nicotinic" CG:GC pair, with (2) indicating the mode of binding of nicotine and (3) of ACh. [BP1 + BP2] illustrates the "muscarinic" GC:GC site, with the fit of muscarine and ACh shown. The O's and N's attempt to indicate the location not of the atoms themselves but of the hydrogen bonds they can form; hence the distance between them is influenced by the bond angles. [Smythies]

GC:CG site can likewise be shown to bind glutamate via its negatively charged O's to the two guanine N's (6 A apart) in the floor of the site. The fact that glutamate can fire any cell may be explained in terms of this model by the observation that both the other two sites (GC:GC and CG:GC) also happen to contain two NH groups about 6 A apart,* to which glutamate can bind by a conjoined ionic bond. In the case of glycine, this short molecule can only bind across the NH and O groups of one base pair. An appropriate prostaglandin to specify the glycine site is dinor F2 α in which the side chain ending in the carboxyl group is two carbon atoms shorter. This will therefore bind to RNA just as E_1 does, except that only 3 base pairs are involved, and only one base pair is exposed in the floor of the lipophilic cup constructed by the prostaglandins. The flat, rhomboidal molecule of strychnine (which blocks glycine sites) closely fits inside this cup and forms a hydrogen bond from its C=O group to the prostaglandin carboxy OH.

These sites are all in the minor groove. The adrenergic site may be located in the major groove. This is suggested by the fact that 2-substituted phenothiazines such as chlorpromazine and perphenazine, which can intercalate into nucleic acids (Ohnishi and McConnell, 1965), can do so only from the side of the major groove. This can locate the long phenothiazine side chain running down the floor of the major groove and bonding by its N,N and OH to three bonding groups on the base pairs – and these bonds must be 3 A apart. This indicates that there can be no prostaglandin on the other side of the RNA, because the minimum possible distance with a prostaglandin attached is 4 A. The NE can bind in the major groove by its phenolic OH's to the uracil O's, its β-OH and NH_2

*These measurements are made from the midpoint of hydrogen bonds formed by these atoms in order to take the bond angles into account.

to the O's on two adjacent phosphate groups. Alternatively, it could bind to the two phosphate oxygens from its ring hydroxyls via a magnesium atom. In this case, its β-hydroxyl and amine groups could bind to elements on the base pairs. A third possible mode of binding utilizes only the NH and O groups on the bases in the major groove. The ring hydroxyls can bind across one base pair (3 hydroxyl H to guanine O; 4 hydroxyl O from cytosine NH; it is more likely that the 3 hydroxyl H is used because 3 O-methylation is used to inactivate the compound). The β-OH now binds to a guanine O and the terminal N to guanine O. Thus the NE receptor could be specified by a CG:GC:GC:GC base pair combination, which is the reverse of the ACh(M) receptor plus an F-type prostaglandin. In all three cases, however, NE could bind to "contracted" RNA and stabilize it in this configuration. In the case of the 5-HT receptor, the fit of 5-HT has already been described. However, the prostaglandin attachment enables us to see how d-LSD and THC bind, and why only d-LSD is active. Owing to the skew in the D ring of LSD and the fixed C—N amide bond, the D ring and diethylamide groupings of d-LSD form a deltoid clump tilted at an angle of some 20° to the plane of the indole ring. In a like manner, the complex ring system of THC forms a delta-shaped clump tilted at an angle of 20° to the plane of the benzene ring. Because of the mode of binding of the prostaglandin side chains to base pairs 1 and 4, these side chains are tilted at an angle of some 20° to the plane of the bases. Thus in the case of both d-LSD and THC, when the π cloud is intercalated between base pairs, the tilted lipophilic system fits exactly into the angle of the prostaglandin molecule, with multiple weak interactions. In addition, the THC hydroxyl can form a hydrogen bond to the adjacent ribose ring oxygen. The three inactive isomers of LSD cannot bind at all, owing to steric hindrance between their diethylamide groups and the prostaglandin. The 5-HT site can bind two molecules of d-LSD or THC. The helix, to bind LSD and THC, must be wound right-handed. An indication that the cholinergic neuromuscular junction may be similarly constructed from a prostaglandin-RNA complex is given by the fact that toxiferine 1, the most potent curarizing agent known, fits precisely into the larger cholinergic receptor (using a type E1 prostaglandin), just as strychnine fitted precisely into the smaller glycine receptor (using a dinor F2 α prostaglandin). Toxiferine 1 binds by its extremely close fit (it is also rhomboidal in shape) two ionic bonds from each of its quaternary N's to prostaglandin carboxyl O's, and two hydrogen bonds from its two hydroxyls to the ribose ring oxygen on base pairs 1 and 4. A large number of agonists and antagonists of these transmitters, as well as compounds

like ouabain, veratridine, and tetrodotoxin, have been compared stereochemically with these postulated receptor sites, and in each case, an unambiguous and close mode of binding to RNA is apparent. The mechanism of action of LSD could potentiate 5-HT effects by binding in the two intercalation sites adjacent to the central site for 5-HT. It could also potentiate cholinergic transmitters, because ACh could still bind, even with two molecules of LSD (or of THC) bound in intercalation sites 1 and 3. But LSD would clearly be incompatible with NE effects, for the former would tend to stabilize the helix in extended position, and the latter would stabilize it in the contracted position. This has been detailed in other publications now in press (Smythies, 1969, 1970). Likewise, NE effects should generally be limited by prostaglandin.

Reserpine also bears a close stereochemical relation to helical RNA (wound *left*-handed, and without any prostaglandin). This suggests that left-handed RNA, or a complex of poly U and ATP, may be concerned with amine channels (or amine storage sites) in granular vesicles and chromatin granules. This may explain the observation by Phillippu and Schumann (1963) that ribonuclease releases catecholamines and ATP, but not protein, from adrenal chromatin granules. Further experimental support for this suggested role of RNA in membrane is given by Namba and Grob (1967) who reported that they had been able to extract a ribonucleoprotein from muscle that binds tubocurarine, which binding is inhibited by ACh. They suggest that ribonucleoprotein may be concerned in the muscle cholinergic receptor. Recently, the excitability-inducing material (EIM) factor that renders an artificial lecithin membrane electrically excitable has been shown to consist of RNA and protein in approximately equal proportion (Kushniv, 1968).

Table 11 summarizes the specification of the receptor sites for various putative transmitters based on the prostaglandin-RNA complex.

It has sometimes been suggested that 5-HT and NE are not transmitters in the sense that ACh or some of the amino acids are, but rather that their function is to modulate other synapses. They have been linked to emotional and reinforcement mechanisms, and it has been suggested that their widespread secretion in the brain may carry the "now print" or "don't print" signals postulated by Livingston (1965).

If we examine our model in the light of this hypothesis, the following mechanisms suggest themselves. In the prostaglandin-RNA complex synaptic receptor, clearly both ACh (or some amino acid) and 5-HT could bind because they are bound to quite different places, and neither sterically hinders the other. The function of 5-HT might be to

TABLE 11
Receptor Sites in the Prostaglandin-RNA Complex

Base Pair	ACh(M)	ACh(N)	Glutamate*	Glycine	NE	5-HT
2**	GC	CG	GC*	GC=CG	GC	?
3	GC	GC	CG*		GC	?
Prostaglandin	20C	20C	20C	18C	0	20C
Groove	minor	minor	minor	minor	major	intercalation
Sense	R	R	?	R	R	R
Torsion	Extended	Extended	Extended	Extended	Contracted	Extended
Antagonist	Atropine	Decameth-		Strychnine	Chlor-	LSD
Explained	Toxiferine 1	onium	–		promazine	THC

*Glutamate should fire any receptor except glycine.
**Base pairs for 1 and 4 may be GC or CG depending on the particular prostaglandin glycine where the base pairs concerned are 1 and 3 (GC or CG).

stabilize the helix and thus inhibit the capacity of ACh to disrupt it. Because the stabilization energy of the RNA helix is mainly provided by the base-pair stacking effect (as noted before), the prostaglandin maintains the helix in an unstable, extended configuration. The intercalation of three molecules of 5-HT would restore the π-cloud stacking, and furthermore, each molecule would bind the two ribose-phosphate chains together by the energy of 3 or 4 hydrogen bonds. The electron-donating capacity of 5-HT might not be concerned in polarizing the RNA base pairs (and so disrupting the hydrogen bonds joining them), but rather with maintaining the semiconductor properties of the RNA polymer that may play some role in the electrical dynamics of the membrane.

Norepinephrine might also equally stabilize the helix, but in the contracted position associated with pore opening in some of the models presented earlier. Thus if 5-HT signalled negative reinforcement, its role, as postulated above, could be to inhibit synapses active at the time, and if NE signalled positive reinforcement (Poschel and Ninteman, 1969), it might potentiate any synapses active at the time, thus promoting conditioning and learning effects.

This mechanism may be closely related to the ATP and Ca^{++} and Mg^{++} systems. The helix would appear to require an ATP-based energy-donating system to re-extend, and the torsion on the helix may be affected by levels of Mg^{++} and Ca^{++}. The configuration of RNA is affected by the ionic environment.

The stereochemical relationship between ouabain, erythrophlamine, and helical RNA suggests the helical RNA may also be concerned in

the active Na^+ pump linked to Na^+, K^+-dependent ATPase. A possible mechanism to effect this might be as follows. If we take the "double spiral chute" model of a membrane channel outlined earlier, one channel could conduct Na^+ out of the cell, and the other, K^+ in. There is evidence that the ATPase is located on the inside of the membrane. If this was located immediately over the inner opening of the "Na^+" channel, its function might be to remove the water of hydration from the Na^+ ion. The hydrated Na^+ ion orders surrounding water with its A-type hydration shell and thus could not pass through a spermine-RNA channel, but the bare Na^+ ion is not hydrated, and this could now run along the "spiral chute" to the outside. Here it would immediately be rehydrated, and thus no Na^+ could pass back into the cell backwards along this pathway. The other channel could conduct the smaller hydrated $K^+(H_2O)_2$ ion (whose B-type hydration shell *disorders* surrounding water) into the cell to replace the Na^+ extruded by the specific ATP base system. This mechanism depends on Mg^{++} and is inhibited by Ca^{++} and ouabain. In addition to acting as an acceptor of water, the Mg^{++} may be required to maintain the helix at the correct degree of torsion to keep the channels open, and the Ca^{++} could induce a different degree of torsion, which would result in closing the channel. The ouabain would fit inside the spiral chute channel and literally plug it, binding by a multiple lipophilic contact and 2 hydrogen bonds to the polyamine struts on the outer wall and by 5 hydrogen bonds to elements on the RNA on the inner wall. Molecular models indicate that erythrophlamine, which has the same pharmacological effect as ouabain, can also block the channel by a similar mechanism. This basic mechanism may be modulated by the ionic changes producing changes in the torsion in the helix, and hence the bore of the RNA-polyamine channels.