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Histamine and GABA

Hydrogen bonds and permeation of vesicles

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Spectroscopic studies on sodium di(2-ethylhexyl)-sulfosuccinate (AOT) inverted micelles, films of AOT and L-α-lysolecithin and on dihexadecyl phosphate vesicles show that histamine and γ-aminobutyric acid (GABA) act differently on these membrane models. Histamine increases the permeability of the membrane to ions through interactions with its polar sites. GABA, on the other hand, prefers self-association to association with the membrane. If these two neurotransmitters are applied jointly, the result is a decrease in the permeating effect of histamine. Possible mechanisms for these processes are discussed.

1. Introduction

Histamine and GABA are two major neurotransmitters. Although the mechanism of their action is not understood in all its details, it is now widely accepted that a key step in this mechanism involves an interaction between the neurotransmitter and a biological membrane (neurone, muscle end-plate,...).

As a result of this interaction the selective ion permeability of this membrane is altered. Consequently, the action potential is either amplified or inhibited depending on the new balance of charges between the internal and external walls of the membrane.

The relationships between the chemical structure of these compounds, or of any neurotransmitter in general, and their biological activity is not obvious. Neurotransmitters may indeed have widely diverse structures. The problems of identifying and defining these relationships are made even more difficult because of the complexity of the structure of the biological membrane.

Membrane models can be very useful in this context. Although they are less elaborate than their biological counterparts they have the advantage of being easy to define and their study can help us to elucidate and identify certain fundamental mechanisms of neurotransmission.

In this paper we shall present the results of a study of the interactions between histamine or GABA and some membrane models by FTIR and electronic spectroscopies.

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Abbreviations: AOT, aerosol-OT, sodium di(2-ethylhexyl)sulfosuccinate. DHP, dihexadecyl phosphate; FTIR, Fourier transform infrared; GABA, γ-aminobutyric acid; LL, L-α-lysolecithin.

2. Experimental

The FTIR instrument used in our experiments was a Nicolet 5DXB spectrometer equipped with a high-sensitivity DTGS detector and high-speed software. The spectra were recorded at a resolution of 2 cm^{-1}.

The FTIR study was carried out in two stages.

(i) We investigated the interactions through hydrogen bonds between histamine or GABA and some membrane models in films. These films were prepared (on AgCl windows) from aqueous solutions of these compounds.

The membrane models used at this first stage were AOT (Aldrich), and L-α-lysolecithin from egg yolk (Sigma) (see ref. 1 for the method of purification of AOT).

(ii) We examined the same interactions in inverted micelles of AOT. In this case samples were prepared as follows: AOT was dissolved in CCl_4 where it formed inverted micelles in the presence of traces of water [2]. We then injected some small known quantities of an aqueous solution containing the neurotransmitter (histamine or GABA) in the organic solution of AOT. FTIR spectra were then recorded using a 0.1 mm CaF_2 cell. In order to discriminate between the effects of the water molecules and those of the neurotransmitter we also studied the FTIR spectra of a similar series of samples where only water was used instead of an aqueous solution of the neurotransmitter.

The electronic spectra were recorded on a Cary-17 spectrometer.

The samples for the purposes of the electronic spectroscopic study were prepared as follows:

All the samples were aqueous. We first prepared a solution of vesicles of DHP (Sigma) by sonicating an emulsion of DHP (49.2 mg) in triply distilled deionized water (15 ml), at a temperature of 80 °C, for about 45 min. During this process we added 450 μl of a 0.1 M NaOH solution and 1.485 ml of a 0.01 M solution of tris(2,2′-bipyridyl) ruthenium(II) chloride hexahydrate (our probe) (Sigma). The volume was brought up to 45 ml. An orange-colored solution containing the DHP single-compartment vesicles was then obtained. At this stage, some of the probe ions are trapped in the internal compartment and the rest are bound to the external surfaces of these vesicles [3].

The ions were then washed off the external surface of the vesicles using a Bio-Rad AG 50W-X2 resin (150–300 μm in diameter). An electronic spectrum of this solution was then taken in the visible ultraviolet region (600–320 nm, band at 453 nm). The absorption of the 453 nm band is proportional to the concentration of the trapped ions.

The neurotransmitter (histamine or GABA) was then added to the prepared solution of vesicles. The amount of probe ions that leaked out of the vesicles could easily be determined by electronic spectroscopy after these ions had been washed off the external surface to which they bind once they diffuse out of the vesicles [4].

For a given concentration X of the neurotransmitter introduced into the vesicle solution the percentage of trapped ions is determined by the simple equation:

$$\rho_X(\%) = 100 \times [A_X/A_0] \quad (1)$$

where A_0 and A_X are the absorptions of the vesicle solution in the absence of neurotransmitter and in the presence of a neurotransmitter at concentration X, respectively.

The experimental errors will be discussed in more detail below.

3. Results and discussion

3.1. Films and inverted micelles

The FTIR study of the above-mentioned films showed that histamine forms hydrogen bonds with the membrane models used, while GABA does not. The latter prefers a self-associated zwitterionic form to the formation of intermolecular hydrogen bonds with the same membrane models.

Fig. 1 shows the FTIR band shifts for a film of histamine in the presence of AOT. The deformation band at 1580 cm^{-1} due to the NH_3^+ group is shifted down to 1574 cm^{-1} in the presence of AOT.

Fig. 2 shows similar results with films of histamine in the presence of a phospholipid. The deformation band is shifted by approx. 15 cm^{-1}

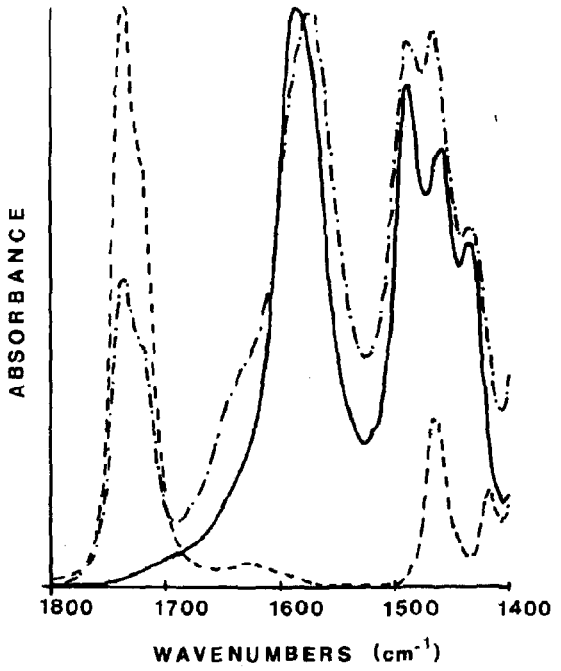

Fig. 1. Partial FTIR spectra of films prepared from aqueous solutions of: (———) histamine, (- - - - - -) AOT, (·-·-·) histamine + AOT.

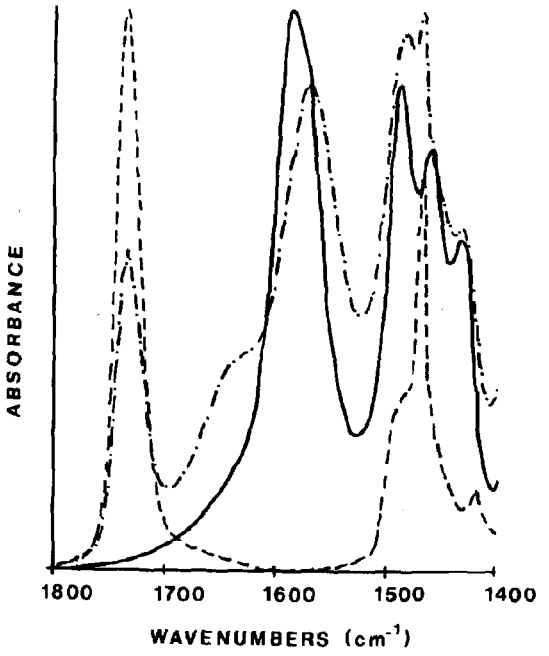

Fig. 2. Partial FTIR spectra of films prepared from aqueous solutions of: (———) histamine, (- - - - - -) L-α-lysolecithin, (·-·-·) histamine + L-α-lysolecithin.

downward from 1600 cm^{-1}.

Examination of other regions of the above-mentioned spectra shows that he characteristic bands of the polar groups of AOT (two bands near 1250 cm^{-1} for the $^{-}OSO_2$ group [5]) and a band at 1247 cm^{-1} for the phosphate group of L-α-lysolecithin are also slightly perturbed.

Since no clear evidence for the existence of histamine–AOT or histamine–L-α-lysolecithin hydrogen bonds with the secondary imidazolic NH group can be obtained from these spectra, we must conclude that these interactions are either nonexistent or very weak.

On the other hand, fig. 3 shows the extreme similarity of the spectra of films of GABA with and without L-α-lysolecithin (the only new bands are those of the L-α-lysolecithin molecule). Except for the L-α-lysolecithin bands themselves, these spectra have no band above 1680 cm^{-1} showing that all carboxyl groups are ionized. The bands observed at 1654, around 1550 and 1380 cm^{-1} are vibrations of the COO^{-} and NH_3^{+} groups.

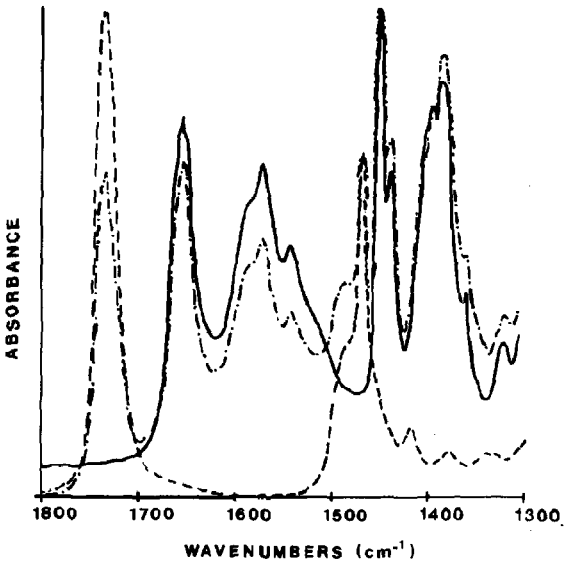

Fig. 3. Partial FTIR spectra of films prepared from aqueous solutions of: (———) GABA, (- - - - - -) L-α-lysolecithin, (·-·-·) GABA + L-α-lysolecithin.

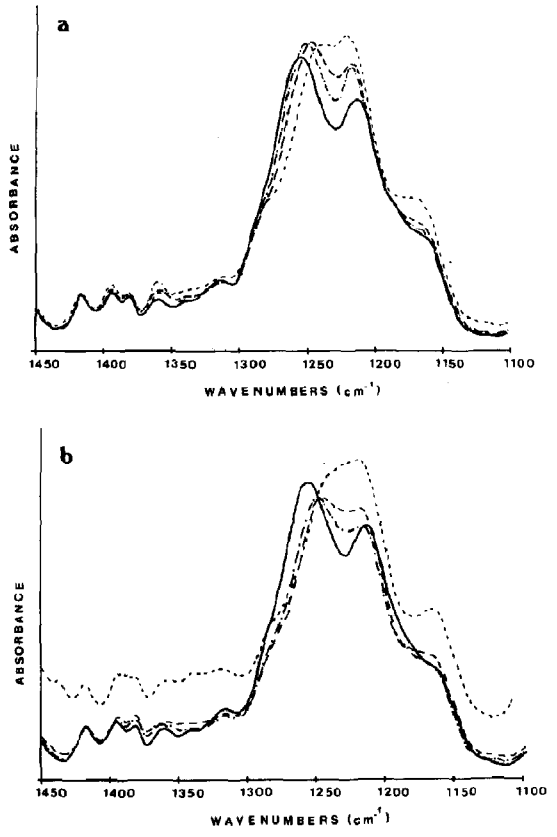

Fig. 4. Partial FTIR spectra (sulfoxide bands) of a 0.1 M AOT solution in CCl_4 to which were added some small known quantities of: (a) water; (b) saturated aqueous solution of histamine. (———) 0.000 ml, (·–·–·) 0.005 ml, (– – – –) 0.010 ml, (· · · · ·) 0.100 ml.

The results concerning the GABA molecule will be discussed further below. It should be pointed out that our results concerning the histamine molecule are in agreement with the assumption that the side chain is responsible for the binding of this type of molecule to the membrane [6].

More evidence for such an interaction is gathered from the spectra of solutions of AOT in CCl_4 (inverted micelles) to which small quantities of an aqueous solution of histamine were added. As is shown in fig. 4, the changes in position and intensities of the sulfoxide bands are different for AOT + H_2O (fig. 4a) and for AOT + histamine + H_2O (fig. 4b). This indicates the existence of AOT + histamine interactions in addition to those of AOT + H_2O.

No such interactions were detected for GABA when it was introduced into a solution that contained inverted micelles of AOT.

3.2. Vesicles

DHP vesicles are expected to be much better models for the study of the dynamics of the interaction between our two neurotransmitters (histamine and GABA) and a membrane, since their bilayer structure approaches that of real biological membranes. Similar vesicles have been used by Nichols [7] and Bangham [8] to study, for instance, the pH gradient-dependent uptake of some neurotransmitters.

The ruthenium complex was used for similar studies in anesthetics [4]. It was chosen as a probe here because it satisfies several experimental requirements for the purposes of this study. It has a very simple and intense spectrum in the visible region. It is small enough to penetrate the vesicles and has a linear permeability with respect to the concentration of a variety of perturbants. This last property enables us to quantitate the permeation potency of our neurotransmitters. We define the PD_{50} as the concentration of neurotransmitter that is required to empty the vesicles of 50% of their initial probe-ion content. Furthermore, a commercially available resin, i.e., Bio-Rad AG 50W-X2, is highly selective for the ruthenium complex and can be used to wash it off the external membrane of the vesicles without damaging the latter. We should add that these conditions are unfortunately not fulfilled by any of the known biologically more relevant ions.

It is also important to point out that this system is very stable and that spectroscopic measurements lead to values of $\rho(\%)$ (determined using eq. 1) that are reproducible within an error of 5–10%. The percentage of trapped ions in solutions of DHP vesicles is still the same (within experimental error) even several weeks after their preparation. Measurements were repeated at least three times for a given concentration of neurotransmitter. When the corresponding values of $\rho(\%)$ were not consistent within the experimental error the whole

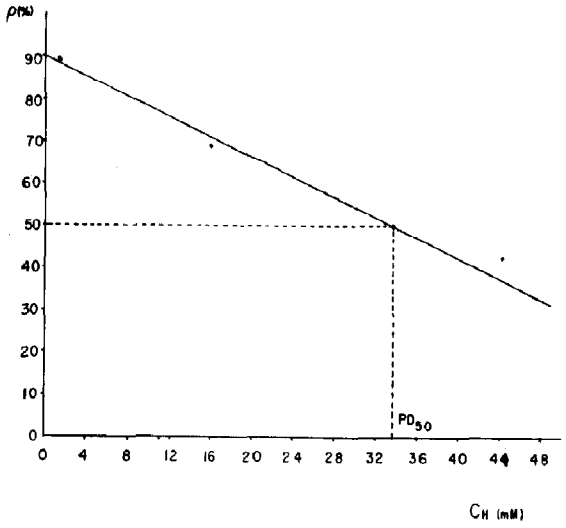

Fig. 5. Effect of histamine on the permeability of DHP vesicles to the probe ions. $\rho(\%)$, percentage of trapped ion; C_H, concentration of histamine; PD_{50}, dose (mM) required to empty the vesicles of 50% of their initial probe-ion content.

series for that given concentration of neurotransmitter was repeated. The mean value of $\rho(\%)$ (for the three consistent results) was then plotted against the corresponding concentration of neurotransmitter. The consistency of the results is of course better (5%) for concentrations of histamine of the same order of magnitude as or higher than the PD_{50}. Indeed, as the number of ions that leak out becomes larger, the error incurred in the experimental determination of the value of $\rho(\%)$ with respect to the 100% point (no perturbing agent added) is of course much smaller.

Fig. 5 shows the results obtained from the electronic spectra of solutions containing DHP vesicles in the presence of histamine. Histamine clearly permeates the membrane (50% of the initially trapped probe ions leak out with a dose of 35 mM histamine).

Although the initial value of $\rho(\%)$ is by definition equal to 100% in eq. 1, extrapolation to zero of the experimental values of $\rho(\%)$ may lead to a value slightly different from 100%. This occurs in fig. 5 but the extrapolated initial value of $\rho(\%)$ is consistent with the 5–10% experimental error margin which takes 'passive leak' into account.

GABA has no effect on the permeability of the DHP vesicles up to its limit of solubility in water.

Before we present the final results of the permeation study we should like to discuss some physicochemical aspects of the permeation process.

The electronic spectrum is recorded after the neurotransmitter has been introduced into the vesicle solution and after the resulting solution has been passed through the AG 50W-X2 resin. When this solution was passed through the resin column for a second time, the absorption of the sample was found to be the same within experimental error (after dilution was taken into account).

Therefore, the permeation of DHP vesicles by histamine or GABA is a transient process and the corresponding $\rho(\%)$ values are given at thermodynamic equilibrium. The equilibrium state is actually reached quite rapidly, probably long before the solution has passed through the resin. Indeed, the results remained unchanged, within experimental error, when the solution was transferred to the resin column immediately after histamine or GABA had been introduced into it or when it had first been left in a closed vial at room temperature for a longer time before it was passed through the resin.

A more quantitative time-course study of permeation might reveal more interesting data. However, it would require a much more sophisticated experimental method where the time between the introduction of the perturbing agent in the vesicle solution and the analysis is minimized.

Therefore, in this study we are looking at the permeation process from a thermodynamic point of view.

A few results suggesting the possibility of interaction between histamine and GABA in biological systems have been published within the past few years [9–11]. This led us to investigate such a possibility in our chemical model.

Fig. 6 shows the results obtained when both GABA and histamine were introduced into a solution of DHP vesicles. We should point out that the values of $\rho(\%)$ (experimentally determined) correspond here to the joint effect of both histamine and GABA on the vesicles. This is due to the fact that the solution was passed through the resin

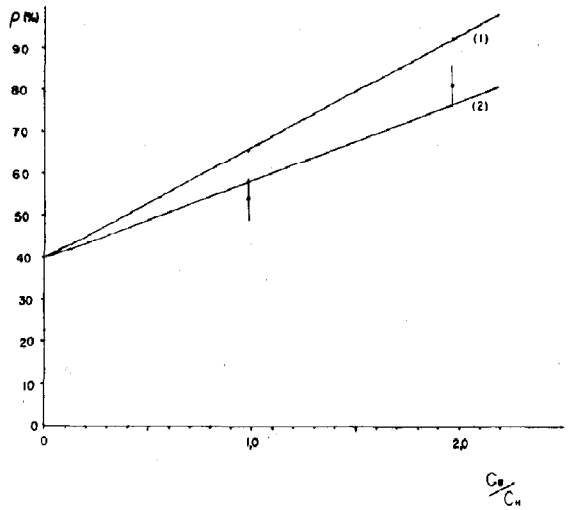

Fig. 6. Joint effect of histamine and GABA on the permeability of DHP vesicles. (1) GABA introduced first, (2) histamine introduced first. $\rho(\%)$, percentage of trapped ions after both neurotransmitters have been introduced; C_G/C_H, ratio of concentration of GABA vs. histamine.

column only once both neurotransmitters had been introduced into the solution.

Surprisingly enough, GABA does have the effect of reducing the permeation potency of histamine. This effect is more accentuated when GABA is introduced into the solution of vesicles before histamine (regression 1) than when it is introduced in a similar solution after histamine has had the chance to exert its effect on the membrane of the vesicles (regression 2).

In this last series of experiments the concentration of histamine was chosen to be at least equal to the PD_{50}, since as explained above, the error margin is smaller in this region. The experimental value of the percentage of trapped ions in the presence of histamine and before GABA is introduced into the same solution is 40%. We emphasize that it is experimentally clear that GABA does reverse the effect of histamine, since the percentage of trapped ions in the presence of GABA is higher than 40% (initial value of $\rho(\%)$ when only histamine is present at a concentration close to the PD_{50}). Even for a C_G/C_H ratio as low as 0.5 the percentage of trapped ions is significantly higher in the presence of GABA. Error bars are displayed on the lower curve of fig. 6 to show that the two curves are significantly different especially for large values of the C_G/C_H ratio. As discussed above, these bars correspond to an error margin of $\pm 5\%$.

These final results lead us to the formulation of the following mechanism of action of GABA and histamine on the vesicles.

When GABA is introduced into a solution of vesicles, it has no effect on these because it prefers to remain in a self-associated zwitterionic form. One can presume that the only way that this molecule would associate with the membrane would be that this membrane has centers of opposite charge density. (The positive center would bind to the carboxylate end and the negative center to the ammonium end of this molecule.)

One possible way for GABA to exert some kind of action on the membrane without binding to it would be that the water molecules and protons of the surrounding medium neutralize the charges on this molecule to allow it to penetrate the hydrophobic part of the membrane by becoming more liposoluble.

Our results do not support the second mechanism but are in perfect agreement with the first. In other words, our results support the idea that GABA is mainly a surface-acting neurotransmitter [6] as all amino acids are expected to be.

The situation is somewhat different for histamine. Indeed, once this molecule binds itself to the polar head of the external surface of the vesicles (as the FTIR results would suggest), the remaining liposoluble imidazole ring can penetrate the hydrophobic barrier of the membrane of the vesicles. By doing so, the histamine molecules exert a perturbation on the entire structure of the membrane, creating interstitial gaps that allow some probe ions to escape from the internal compartment. The process would continue until the whole system reaches a state of equilibrium where the histamine molecule becomes a part of the structure of the vesicle and the probe ions no longer have the chance to escape.

How can GABA affect the potency of histamine if it is introduced into the solution once histamine has exerted its effect on the membrane of the vesicle? If it is quite clear experimentally

that GABA does reverse the effect of histamine it is not as clear why it tends to do so. There is indeed no other way of explaining the fact that the effect of histamine is reversed by the addition of GABA to the solution than by the assumption that the ions diffuse back into the internal compartment of the vesicle as a result of some kind of interaction between GABA and the vesicle. Along the same line of thought, one can assume that, at this stage, GABA can perturb the external surface of the vesicle since this new vesicle, which now contains histamine as an integral part of its structure, does present on its surface some centers of opposite charge density to that of the DHP polar heads (due to the histamine amino groups that are bound to some of these polar heads). This allows the probe ions that leaked out during the first step of the experiment to diffuse back into the vesicle.

This may seem to be in contradiction with the laws of thermodynamics but one can argue that it is not necessarily so. Indeed, if one assumes, as previously discussed, that when histamine acts on the vesicles a new entity (that could be referred to as the vesicle-histamine complex) is formed and it is this new entity that now interacts with the GABA molecules that are subsequently introduced into the solution. Therefore, it is the thermodynamic properties of this new entity and its interaction with GABA that govern the diffusion of the probe ions (present in the solution, most likely bound to the external surface of the vesicles) back into the vesicles.

If GABA is introduced into the solution of the DHP vesicles before histamine then the overall observation can be explained by a direct interaction between these two molecules that would decrease the efficiency of histamine in acting on the vesicles (the histamine molecule does present centers of positive and negative charge density [12]). It is not so surprising that in such a mechanism GABA is slightly more efficient in decreasing the potency of histamine than in the previous case (regression 2 compared to regression 1 in fig. 6).

Such mechanisms of action are in agreement with the assumption made by biologists [6]) that histamine belongs to a second class of neurotransmitters that do interact with the external surface of the membrane (as amino acids do) but also exert their action through the hydrophobic barrier of this membrane.

It is important to emphasize here that had histamine belonged to a third class of neurotransmitters that are active once they completely diffuse through the membrane, GABA would never have the chance to reverse its effect, since the external surface of the vesicle, after the introduction of histamine, would be the same as that of a vesicle that did not interact with histamine. Furthermore, since GABA cannot diffuse through the membrane, no other interaction with the histamine molecule would be possible.

4. Conclusions

It is believed that this preliminary spectroscopic study provides pieces of evidence on the following points:

(i) GABA is a surface-acting neurotransmitter while histamine is a transmembranal neurotransmitter.

(ii) Histamine binds to the external surface of the membrane by the amino group of the lateral chain.

(iii) GABA can modulate or reverse the effect of histamine on the permeability of vesicle membranes.

(iv) The effect of neurotransmitters on membrane permeability to ions was shown to be a rather complex phenomenon even without the presence of such elaborate structures as receptors and ion channels that real biological membranes have. It is interesting to note that even without the presence of these structures the mechanisms of action of our two neurotransmitters can already expected to be quite different.

Our model is intended for investigation of some fundamental physicochemical processes involved in neurotransmission in the broadest sense. It seems to be suited to mimicking the dynamics of the entrance of neurotransmitters into the brain through the blood-brain barrier (we thank one of the referees for making this suggestion).

This type of approach may help to elucidate the role of receptors and ion channels in the phenom-

enon of neurotransmitters (from a physicochemical point of view) with the use of vesicles that could possibly include such structures.

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