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REVIEW ARTICLES

In the memory of Dr Olga P. Yurchenko—our colleague in the studies of intracellular transmitter receptors

On the Intracellular Transmitter Reception[1]

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Abstract—The idea on the possibility of intracellular neurotransmitter receptor localization exists already more than half a century, however it remains disputable until now. The data on such neurotransmitter receptors' localization in unicellular organisms, early (pre-nervous) embryos and in adult cells, including neurons are summarized in the present paper. These data were obtained both using pharmacological experiments with pairs of hydrophilic and lipophilic analogues of transmitter receptor ligands, by direct ligand microinjection into the cells, and also as by labelled ligand binding and using electrophysiological methods. The data on the intracellular localization of transmitter receptors provokes to critically evaluate the current understanding of the origin of transmitter substances and corresponding receptors. It is suggested that they were formed as the result of the evolution of function of systems that originally were coupled to the processes of intracellular syntheses but not to cellular interactions.

Keywords: neurotransmitters, intracellular receptor, early embryos, protozoans, neuron, evolution of functions

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The fifties of XX century were the period of intense development of neurotransmitter researches, elaboration of electrophysiological methods and first sets of pharmacological tools in this field. The chemical signal transduction discovered in neuromuscular heart preparation [1] further was treated as neurological mechanisms, and it is the direction where main efforts in world science were concentrated. The neurotransmitters themselves were described as the attribute of neuronal and neuro-muscular interactions only.

However, the facts were soon discovered and corresponding opinions started to form that challenged the exclusive association of chemical transmitters with neurons. The first known fact in this field was that the acetylcholine and acetylcholine esterase are present in the sea urchin gametes and early embryos [2]. However, this phenomenon was not clearly explained by the Author except weak suggestion that these substances are stored for future use.

The dissident views on the origin and function of neurotransmitters arose in the Lab of Physiology headed by Prof. Kh.S. Koshtoyants (former Institute of Animal Morphology, Acad. Sci. USSR) at the same period. The style of this Lab was determined by the personality and approaches of its leader who actively developed the ideas of comparative and evolutionary physiology, ascending to investigations of Prof. I.M. Sechenov. Memoirs of Lab's members a generally consistent that already at the end of fifties the idea was in the air that synaptic transmission arose on the basis of evolutionary ancestral intracellular mechanisms of the regulation as a result of the change of their functions during philo- and ontogenesis. It is now hard to say who was the author of this formula further persisted in the Lab of Embryophysiology, headed by Prof. Gennadii A. Buznikov but this very opinion clearly ruled the direction of his researches.

The first experimental data on the functional activity of neurotransmitter substance in the embryogenesis were obtained in the Lab of Physiology by Buznikov and Manukhin during the expedition to White Sea Biological Station of M.V. Lomonosov Moscow State University. The circumstances of this work were described by its authors in different ways. According to Buznikov' version they were sent to the expedition by their scientific advisor Prof. Koshtoyants with the clear task to find the model for the demonstration of the prenervous functions of serotonin,

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whereas Manukhin described it as follows (personal communication): "We bothered with the batch of nudibranch eggs and somebody suggested to drop serotonin on it." This compound was brought by Koshtoyants just before from foreign visit and piece of it was given to expedition members. Anyway, this experiment was carried out and have shown the effect of serotonin on the larval motility [3].

During some next years the studies in this field were concentrated on the elaboration of suitable models for the researches of prenervous functions of neurotransmitters in the embryogenesis, in particular, simple and useful system for the determination of the functional activity of neurotransmitter antagonists in the fertilized sea urchin eggs that allow quick and mass experiments. Their duration was limited by the growth rate of echinoderm embryos—about 1 cleavage division per hour. These studies have shown that antagonists of serotonin, catecholamines and acetylcholine have embryostatic activity (see [4]).

Evidently, the idea on the intracellular embryonic functions of neurotransmitters was renewed at the beginning of seventies of XX century when the set of neuropharmaca was elaborated, especially pairs of lipophilic and hydrophilic analogues that significantly differ in the permeability into the cells of sea invertebrate embryos. The first research using series of indole derivatives that differ in their lipophily have shown direct dependence of embryostatic effect on the lipophily of these compounds in classical object—cleavage divisions of sea urchin embryos [5]. Later the same approach was used in the model of cellular interactions in the intact sea urchin embryos, where easily penetrating the cell lipophilic substances evoked the formation of dwarf embryos more effectively than their hydrophilic analogues [6].

The experiments on the direct administration of neurotransmitter antagonists into the cells of clawed frog *Xenopus laevis* became the next step in these studies participated by Prof. Turpaev [7]. It was shown that the microinjection of the antagonist of β-adrenoreceptors propranolol evoked the specific temporal blockage of cleavage divisions that could be weakened by addition of adrenaline as distinct from total absence of the effect of the addition of propranolol to the extracellular medium. Similar results were obtained with m-cholinoreceptor antagonist atropine.

Additional arguments in favor of intracellular localization neurotransmitter receptor link in the regulation of the processes of cleavage divisions were obtained in the experiments with labelled ligands of β-adrenoreceptors. The specific binding of [^{3}H]-dihydroalprenolol (K_D 3 × 10^{-9} M) and [^{125}I]-iodocyanopindolol (K_D 1.5 × 10^{-9} M) was found in the microsomal and mitochondrial fractions of *X. laevis* embryos during cleavage divisions [8]. Similar results were obtained with labelled ligand of m-cholinoreceptors [^{3}H]3-quinuclidinyl benzylate in microsomal fraction (Shmukler, Grigor'ev, unpublished data).

The ultracytochemical study revealed the localization of adenylate cyclase mainly in the membranes of endoplasmic reticulum [9] that lead to suggestion that this structure is the most probable localization of intracellular neurotransmitter receptors.

Thus, the data accumulated by various methods clearly evidence in favor of intracellular localization of the receptor link of neurotransmitter process during early embryogenesis, at least, in echinoderms and amphibians, moreover of several neurotransmitters simultaneously. At this very period the idea that such localization is the specific feature of neurotransmitter processes in the early embryogenesis became sacred paradigm. The relative effective distances of neurotransmitters and second messengers [11] served as the ground for "ostensible abundance" of signal molecules in the embryonic cell [10]. These distances amount to about 20 μm for inositol triphosphate and about 3 μm for Ca^{2+}, whereas the size of the eggs is greatly larger but there are practically no limits of serotonin effective distance.

About the same time several studies were published on the possibility of intracellular localization of some neurotransmitter receptors in the cell of adult organisms. In particular, the inhibition of histamine binding by its antagonist have shown the possible role of this neurotransmitter as the intracellular messenger, triggering the platelet aggregation [12] and the formation of granulocytes/macrophage colonies (CFU-GM) by the normal bone marrow human and mouse cells, formation of leukemic colony (CFU-L) by the cell line of mouse leukemia (WEHI 3B) and the formation of the colony by the bone marrow cells of patients with chronic myeloid leukemia (CML) via inhibitory influence on histamine formation de novo and disturbing the interaction between histamine and its intracellular binding sites [13].

Probably, inspired by the success of the works in direct evidence of the intracellular localization of neurotransmitter receptors in the early amphibian embryos Prof. Turpaev initiated and then supported the series of studies carried out mainly by Dr Olga Yurchenko who previously dealt with the interaction of the neurotransmitters in neuronal processes [14].

It was shown that the intracellular perfusion with neurotransmitters influences the acetylcholine responses of molluscan neurons, i.e. serotonin added to both intracellular and extracellular experimental media decreased the responses to acetylcholine in some isolated neurons of parietal and visceral ganglia of *Lymnaea stagnalis*. In some other neurons serotonin added to the intracellular solution increased the response to acetylcholine, whereas added extracellularly evoked the opposite effect in the same cells [15]. In other object—voltage-clumped neurons R2 of the abdominal ganglion of mollusc *Aplysia depilans* intra-

cellularly injected dopamine increased the amplitude of inward and outward currents, recorded in response to acetylcholine application. The addition of dopamine to the external perfusion solution evoked the decrease of the response to acetylcholine [16]. These data evidence the modulatory effects of serotonin and dopamine on the cholinergic synaptic transmission in the molluscan nervous system suggesting the presence of intracellular neurotransmitter receptors here.

Unfortunately, non-scientific circumstances stalled this interesting and promising line of the researches, and no its development was found in the contemporary literature.

In parallel the researches of the role of neurotransmitters in the unicellular organisms began, that had a great significance for the understanding of the evolution of functions of these substances [17], see also the review [18]. Concerning the neurotransmitter reception in protozoans the interaction of transmitters with membrane receptors cannot be ruled out [18], while the presence of D_1-dopamine receptors localized in the endoplasmic reticulum and endosomes [19] was shown, similar to that found in early sea urchin embryos (see above) in which also membrane receptors were discovered [20].

During last decades we get back to the matter of the intracellular localization of the transmitter mechanisms in the early embryos of sea urchin in the connection with elaboration of new pharmacological tool—the conjugates of the neurotransmitters with such fatty acids as arachidonic and eicosapentaenoic ones, that have far more abilities to penetrate the cells than their hydrophilic analogues [21]. The usage of serotonin or dopamine arachidonic derivatives have shown, on the one hand, their far more pronounced protective action against embryostatic neuropharmaca as compared to these neurotransmitters themselves, and on the other hand, in contrast to previous data, the absence of clear pharmacological specificity of the receptor link in sea urchin embryos where both arachidonoyl-serotonin and arachidonoyl-dopamine have practically equal protection effect against the same transmitter antagonist [22]. At the same time antagonists of serotonin and dopamine receptors differ greatly by their effects on the state of tubuline cytoskeleton (Shmukler, Nikishin, in press).

Thus, we can state that a great amount of the data was accumulated to date that in various ways evidence the existence of intracellular neurotransmitter receptors to serotonin, dopamine, histamine etc in unicellular organisms, in the early embryos and in the definitive cells of adult metazoans, i.e. on the universal character of this phenomenon. Within this context it is worth to return to the beginning of present paper—to the idea on the origin and original functions of the neurotransmitters in the evolution.

The existing concepts of the formation of transmitter systems anyway assume their connection with neuronal synaptic process that leads to the psychological trap preconditioned by the semantic of the term "neurotransmitter" although the internal contradiction here is evident.

The paradigmatic question "Why do neurons have different transmitters when any one transmitter could in fact mediate all the required electrical signals?" [23], repeated in an assertive form by D.A. Sakharov: "every physiologist who overcame through science of synaptic interactions earlier or later come to the conclusion that single transmitter would be quite enough for the nervous system" [24], could be answered that neuronal information transfer itself could really be organized using the single neurotransmitter, if it arose just at the moment of the formation of nervous system and specially for the transmission of nervous impulse from one cell to another.

However, it should be recognized that all the cells of the organism at the period of nervous system formation already had the long prehistory and accumulated mechanisms that were fitted for this important but not unique developmental process. Therefore, the existing concepts of the multiplicity of neurotransmitters are suitable only for the explanation how these mechanisms could be tuned during the evolution of multicellular organisms. The mere existence of the multiplicity of neurotransmitters might be considered not only coming from their intended purpose in the organism but on the basis of their origin.

The starting point for further disclose is the postulate of phylogenetic antecedence of ontogenetically primary functions of the transmitters, i.e. ones that are realized during early embryonic development. Taking into account multiform activity of transmitter systems in Protozoans and at the prenervous developmental stages of Metazoans it is logic to suggest that phylogenetically transmitters arose far before the formation even of the simplest nervous system and moreover even before multicellularity. During phylogenesis the transmitter functions were repeatedly modified that reflect the changes in the ontogenesis we can see at contemporary stage of evolution. The intracellular transmitter receptors and systems of intracellular transmitters in the early embryos are the most ancient form of the organization of signaling and were probably the evolutionary predecessor of corresponding definitive systems. The evolution after Francois Jacob is the tinker [25], meaning it invents nothing except transformation of already existing mechanisms. Thus, we return to the idea on the origin of the transmitters—derivatives of essential amino acids tryptophan and tyrosine—as the signal substances, being the probe of the processes of protein synthesis [10].

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