Perspectives on Developmental Neurobiology, 1998, Vol. 5. pp. 469–480
Reprints available directly from the publisher
Photocopying permitted by license only

© 1998 OPA (Overseas Publishers Association)
Amsterdam N.V. Published by license
under the Gordon and Breach
Science Publishers imprint.
Printed in Malaysia.

Functional Coupling of Neurotransmitters with Second Messengers during Cleavage Divisions: Facts and Hypotheses

YURI B. SHMUKIER and GENNADY A. BUZNIKOV*

Laboratory of Embryophysiology, N. K. Koltzov Institute of Developmental Biology, Russian Academy of Sciences, 117808, Moscow

Current data on the role of classical neurotransmitters as multiple and multifunctional regulators of early embryogenesis are reviewed. It is shown that these developmental regulators are coupled with second messengers. Peculiarities of this prenervous coupling emphasized and are used as the basis for discussing the problem of the evolutionary origin of cell regulatory systems.

Keywords: Neurotransmitters, second messengers, early embryogenesis, evolution

INTRODUCTION

Neurotransmitters, at least the classical ones, such as acetylcholine (ACh), serotonin (5-HT), dopamine (DA), noradrenaline (NA), and adrenaline (A), function throughout metazoan development. This concerns also gametogenesis and early embryogenesis, i.e. stages when neurons have not yet developed. It is unlikely that these multifunctional regulators operate in the same way during prenervous and neuronal periods of ontogenesis. In other words, their functions should change during development. There are good reasons to believe that these changes are cyclic, with periodicity from gametogenesis to the adult state and to gametogenesis again, and that prenervous neurotransmitter functions are phylogenetically the oldest in this cycle.

Evolutionarily, these functions precede all other neurotransmitter functions in adult organisms, including synaptic neurotransmission, the most recently developed function [1]. Therefore, one-cell or cleaving embryos contain, in addition to the genetic program of ontogenesis, the complete regulatory machinery ensuring both their existence and development. Hence, such machinery can be regarded as a precursor of the definitive regulatory systems.

Certain results obtained with early embryos suggest that, during prenervous as well as neuronal stages of development, neurotransmitters realize their signaling role via functional coupling with second messengers (for review see ref 1, 2). Such data are insufficient for comparing them in detail with corresponding data on differentiated cells. However, it is apparent that the events considered are more or less different in early embryos compared with adult metazoans [1,2]. Peculiarities of neurotransmitter coupling with second messengers in early embryos are very interesting *per se*, and in relation to a general problem concerning

*To whom correspondence should be sent (Prof. Gennady A. Buznikov, N. K. Koltzov Institute of Developmental Biology, 26 Vavilov St., 117808 Moscow, Russia. Phones: (7095) 135-00-52; (7095) 135-30-55; Fax: (7095) 924-53-91 for 135-80-12; E-mail: buznikov@ibrran.msk.su)

phylo- and ontogenetic changes in inter- and intracellular signaling. This paper reviews data concerning these issues and discusses the theoretical background for subsequent investigations. In addition, the origin of first and second messenger signaling pathways during phylogenesis is discussed.

NEUROTRANSMITTER SYSTEMS IN ONE-CELL AND CLEAVING EMBRYOS

The one-cell embryo is a virtually perfect autocrine system, because it contains all signal substances (first and second messengers), their targets, and all the machinery providing for signal transduction and spatio-temporal organization for appropriate signaling [2-5]. Cells of the cleaving embryo (blastomeres) retain these features, but begin to interact with one another: prenervous neurotransmitters can exit from one blastomere to the extracellular space and interact with receptors of the other blastomere. We believe that this applies at least to animals with so-called regulatory development (echinoderms, vertebrates, etc.). In organisms with mosaic development (polychaetes, molluscs, ascidians, etc.), prenervous neurotransmitter processes may be different to some extent, but corresponding information is insufficient for productive discussion [1,2].

Neurotransmitters

In early embryos, neurotransmitter synthesis proceeds along the usual pathways, but at unusual sites. Thus, biogenic monoamines, especially 5-HT or 5-HT-like substances, and, probably, ACh are synthesized in the yolk [2,6-8].

Another unusual, but general, trend is coexistence of several neurotransmitters within the zygote or an individual blastomere. This applies to polychaetes, echiurides, molluscs, echinoderms, teleosts, amphibians, birds and mammals. For example, functionally active ACh, dopamine (DA), noradrenaline (NA), adrenaline (A), 5-HT and its analogue tryptamine (T) were found in one-cell and cleaving embryos of sea urchins. Interestingly, coexistence of several neurotransmitters was demonstrated for protozoans as well [1,2,9]. This fact appears quite unusual, and we shall discuss it in the final section of this article.

Receptors

Intracellular receptors

The intracellular (cytoplasmic) localization of neurotransmitter receptors (or their functional analogues) involved in control of cleavage divisions was demonstrated for echinoderms and amphibians using various methods, including radioligand binding [10], microinjection of neurochemicals [11], comparison of cytostatic or cytotoxic activities (blockade of cleavage divisions or cell death, correspondingly) in homologous series of 5-HT antagonists with different lypophility [2], etc. There is also some indirect evidence for localization of these receptors to endoplasmic reticulum membranes [2,12].

The nature of intracellular receptors remains unclear. Moreover, it is possible that, in certain cases, they are not true receptors, but rather binding sites on intracellulular organelles. Thus, cyproheptadine (a cytostatic 5-HT antagonist) rapidly destroyed cortical microfilaments in both intact and permeabilized one-cell sea urchin embryos. This specific effect might be due to the direct binding of this compound to cytoskeletal elements [10]. This concept agrees well with data on direct 5-HT binding to cytoskeletal actin and direct connections of neurotransmitter receptors with the cytoskeleton [13,17].

Intracellular localization of all these receptors correlates with their well-manifested pharmacological peculiarities that do not correspond to those of any known type or subtype of 5-HT-, adreno-, or cholinoreceptors [2,3,18,19]. Moreover, these receptors can be highly sensitive to ligands that are inactive in classical pharmacological models. For example, 6-hydroxytryptamine, an inactive 5-HT isomer, can protect sea urchin embryos from the effects of cytostatic 5-HT antagonists to the same degree as 5-HT itself. These cytoplasmic receptors apparently lack any stereospecificity [10,12]. Finally, such receptors seem to be incapable of incorporation into the outer cell membrane [1].

Experiments have also provided evidence for intracellular localization of 5-HT receptors involved in blastomere adhesion in sea urchin embryos [20] and in "supersensitivity" phenomenon [2]. As to the latter, early embryos of certain echinoderm species are 200 to 1000 times more sensitive to very lipophilic antagonists of ACh, 5-HT and catecholamines than embryos of other species. Receptors for such superactive drugs are different from those involved in the control of cleavage divisions.

Surface membrane receptors

Neurotransmitter receptors located on the outer cell membrane have been found in early embryos of various animals. Evidence for the presence of membrane 5-HT receptors in one-cell and cleaving sea urchin embryos has been provided by pharmacological experiments [21,22]; Buznikov, Shmukler, Rakic, and Whitaker, in preparation) and in studies on binding of [H^3]-8-OH-DPAT, a labeled 5-HT_{1A}-receptor ligand [22]. Fluorescent antibodies were used to detect typical muscarinic cholinoreceptors in cell membranes of unfertilized sea urchin eggs [23]. In fertilized eggs, these receptors are substituted by nicotine receptors [23-25]. Certain results of our pharmacological experiments (Buznikov, Shmukler, Rakic, and Whitaker, in preparation) confirmed the presence of nicotinic receptors on outer cell membranes of early sea urchin embryos. These receptors appear to be more similar to receptors of differentiated cells [1], than to intracellular receptors.

Developmental Specificity of Neurotransmitter Functions

In early embryos, the classical neurotransmitters perform two main functions: they participate in triggering and regulating cleavage divisions and in interactions between blastomeres.

To obtain acceptable evidence for the role of any neurotransmitter in these functions, it is necessary to prove the following: (i) the presence of this neurotransmitter in one-cell and cleaving embryos, (ii) the ability of certain antagonists to inhibit or block the process under consideration, and (iii) the ability of the neurotransmitter itself to suppress or abolish such an effect of corresponding antagonists [2,3,19].

With respect to cleavage divisions, these requirements have been satisfied for 5-HT, T, and ACh in echinoderms, polychaetes, nudibranchs, amphibians, and mammals; corresponding data on catecholamines were obtained with sea urchins and amphibians. However, the actual functions of various neurotransmitters as regulators of cleavage divisions remain virtually unknown. In fact, morphological abnormalities caused by antagonists of different neurotransmitters in intact echinoderm embryos appear to be similar. Moreover, 5-HT somewhat protected embryos against the cytostatic effects of ACh and catecholamine antagonists, whereas A and NA protected to some extent against such effects of 5-HT antagonists [2]. Experiments with more adequate models, permeabilized one-cell embryos of sea urchins, in particular, showed that indolylalkylamines and catecholamines have an antagonistic action on cortical microfilaments, their target during cytokinesis [10,18].

Our studies showed that 5-HT or 5-HT-like substances specifically participate in the process of blastomere adhesion in sea urchins [20]. Only 5-HT antagonists can disturb this process, whereas antagonists of other prenervous neurotransmitters are ineffective. Hence, it is probable that regulation of blastomere adhesion, in contrast to cleavage divisions, is the function of serotonergic mechanisms only.

Blastomere adhesion is a prerequisite for subsequent direct interactions between blastomeres. These interactions proceed with the specific involvement of 5-HT-like substances (5-HT or T) that probably operate via cell membrane bound receptors [4,22,26]. According to preliminary data, ACh can function as a 5-HT antagonist in these interactions (Buznikov, unpublished).

Dividing blastomeres form an intercellular compartment where 5-HT or 5-HT-like substances

accumulate [27]. The presence of 5-HT receptors at the contact membrane provides for the formation of a protosynapse. The latter is a bilaterally symmetrical structure in which both cells simultaneously serve as the source of interblastomere transmitter and its target. This contact zone also obstructs diffusion of 5-HT (or T) away from the intercellular space, thus ensuring its relatively high concentration there [22]. With regard to the one-cell embryo as an autocrine system (see above), it is possible that cytokinesis, release of neurotransmitter(s) from the sister cells, and appearance of corresponding receptors on cell membranes are exactly the events whereby this (semi)autocrine system is formed. On the one hand, these early specialized mechanisms of intercellular signaling may represent ontogenetic (and phylogenetic—see below) precursors of neuronal synapses. On the other hand, these mechanisms may provide for cell differentiation and corresponding restriction of developmental potential.

There is certain evidence that 5-HT, ACh, and their antagonists (mainly nicotinic receptor blockers) have specific effects on calcium ions transients and membrane transporters in one-cell and cleaving sea urchin embryos ([28]; Buznikov, Koikov, Shmukler, and Whitaker, in preparation) Corresponding data will be considered in more detail below.

Concerning organization of the prenervous neurotransmitter processes, it was already noted that 5-HT, ACh, and catecholamines participate in specific ways in regulation of cleavage divisions throughout each cell cycle. The functional activity of 5-HT in blastomere adhesion and interactions seems to be transiently expressed. In general, the developmental dynamics of these neurotransmitter functions appears to be very complicated. These functions may disappear and reappear, certain neurotransmitters may act as antagonists or synergists, depending on the function they perform, and their functional antagonism may transform into synergism and *vice versa* during different phases of development [1,2]. Likewise, the functional coupling of prenervous neurotransmitters with various second messenger systems is very dynamic and complex, as discussed below.

FUNCTIONAL COUPLING OF NEUROTRANSMITTERS WITH SECOND MESSENGER SYSTEMS IN ONE-CELL AND CLEAVING EMBRYOS

Virtually all known second messenger systems have been found in early embryos of various species [29-41]. They do not differ significantly from corresponding systems of the adult organism. Remarkably, the fact that these systems of embryonic cells can be under neurotransmitter control has received very little attention. For some investigators, however, it was apparent that prenervous neurotransmitter actions must be mediated by second messengers, as in differentiated cells [2,4,26,29,42]. Moreover, prenervous neurotransmitters appear to be involved in the control of the same processes that are known to be regulated by second messengers.

The phosphoinositide-diacylglycerol-calcium signaling system controls a number of developmental processes [33,34]. In particular, oscillations of inositol triphosphate (IP_3) and free calcium ion levels in sea urchin embryos correlate with such events as fertilization, pronucleus migration, nuclear envelope breakdown, mitotic metaphase-anaphase, and the first cytokinesis [38,43]. Similar functions are attributed to the adenyl cyclase system [44,45]. Indeed, several authors have demonstrated a definite correlation of cAMP levels and corresponding enzyme activities with cytokinesis during cleavage divisions [46-48]. Apparently, both of these second messenger systems are functionally active in embryonic cells. Here, we shall limit ourselves to discussing the relationship between these systems and prenervous neurotransmitters.

First, results related to this problem were obtained when we found that dibutyryl-cAMP and papaverin, a phosphodiesterase inhibitor, act similarly to 5-HT, imitating interblastomere signaling in half embryos of sand dollar *Scaphechinus mirabilis* [26,49]. Moreover, dibutyryl derivatives of cyclic nucleotides and adenyl cyclase activators weakened the cytostatic effects of 5-HT antagonists in sea urchin embryos and the beta-adrenergic blocker propranolol in *Xenopus* embryos [2,11].

Other researchers have obtained more direct evidence for prenervous neurotransmitter coupling with adenyl cyclase [29,30,50,51]. These experiments showed that 5-HT antagonists and DA itself affect adenyl cyclase activity in sea urchin eggs and early embryos. This enzyme can be activated or inhibited through D_1- and D_2-receptors, respectively, and different G-proteins probably participate in this process. It is noteworthy that adenyl cyclase activity in cleaving sea urchin embryos is observed both within blastomeres (at the endoplasmic reticulum and membranes of yolk granules) and on their surface membrane [36]. This is in good agreement with data on the putative localization of prenervous neurotransmitter receptors involved in control of cleavage divisions and early cell interactions [1,2,22,26]. The transient presence of active adenyl cyclase at the contact membranes between dividing blastomeres completes the general picture of the above-mentioned protosynapse as a phylogenetic and ontogenetic precursor of neuronal synapses.

The first data on the interactions of prenervous 5-HT and ACh surface membrane receptors with the phosphoinositide and diacylglycerol systems were obtained in early sea urchin embryos *Lytechinus pictus* ([28]; Buznikov, Koikov, Shmukler, and Whitaker, in preparation) There is good reason to believe that these interactions are important for the control of Ca^{2+} influx [28,52]. Data on the presence of L- and T-type Ca^{2+}-channels in early sea urchin embryos [53] and in eggs and early embryos of ascidia *Ciona intestinalis* [54,55] support this hypothesis. The developmental dynamics of Ca^{2+}-channel activity and distribution correlates with changes in sensitivity of sea urchin embryo cells to 5-HT antagonists. In particular, L-type channels disappear at the vegetal pole of 16-cells embryo of sea urchin, i.e. in micromeres [53] that have a significantly decreased supersensitivity to 5-HT antagonists [2]. The initial results suggest a connection between the embryotoxic effect of neurotransmitter antagonists and a sharp increase (to lethal levels) in cytoplasmic Ca^{2+} concentration (Buznikov, unpublished). There is also evidence that activation of $5HT_2$ receptors and G-proteins in hamster eggs stimulates phosphoinositide hydrolysis that leads to IP_3 production and increased intracellular Ca^{2+} levels [56]. In light of these data, it is desirable to directly test the ability of 5-HT to affect permeability of Ca^{2+}-channels, as well as release of Ca^{2+} from intracellular stores.

Thus, the available data, although limited, allow us to speculate that neurotransmitters and second messengers interact in the cells of early embryos (Figure 1). The presence of both first and second messengers inside the same cell seems superfluous, but only at a cursory glance. We can immediately refer to various facts related to this question. For example, second messengers are effective at relatively short distances (in the case of IP_3, no more than 20 μm, whereas the diameter of a sea urchin egg is about 100 μm or even greater). Calcium ions act within strictly limited domains [57] as a "promiscuous messenger" capable of activating a number of effectors [58]. Some other second messengers (diacylglycerols, arachidonic acid, etc.) can be "promiscuous" as well.

Judging from the effects of A and cAMP microinjected into *Xenopus* blastomeres [59], we suggested that A, which merely accelerates the cleavage furrow formation, is a more "targetted" messenger than cAMP which causes a diffuse "surface bubbling" effect (see also [60]) Apparently, local neurotransmitter receptors confine the cytokinetic signal spatially, whereas more uniformly distributed receptive structures for second messengers respond to a number of signals of various origin. If neurotransmitters in one-cell and cleaving embryos act through corresponding second messenger systems, all peculiarities of their functioning should become apparent at the level of these systems. Coexistence of several neurotransmitters in zygotes and blastomeres, combined with multifunctionality of each neurotransmitter, could result in numerous variants of their coupling with second messengers. There are two groups of such variants corresponding to intracellular or plasma membrane levels for functional coupling of prenervous neurotransmitters with second messengers. Such coexistence of two coupling levels seems to be unusual for normal (non-malignant) differentiated cells.

During recent years, many components previously regarded as specific for the cell surface membrane have been found on intracellular membranes. This concerns cyclases [36], components of the phosphoinositide system [61,62], various ion (including calcium) channels [34,63], G-proteins [64] and intracellular neurotransmitter binding sites mentioned above. The integral complexes for receiving and transducing such signals have been detected on membranes of the nucleus [65,66], mitochondria [67,68], endoplasmic [69], and sarcoplasmic reticulum [70]. Apparently, such intracellular complexes exist in cells of early embryos as well. This supports the idea of general uniformity of known definitive and prenervous regulatory mechanisms.

SOME CONSIDERATIONS ON THE GENESIS OF MESSENGER SYSTEMS

We can define the status of neurotransmitters and second messengers of early embryos as "ostensible redundancy". A long-standing concept was that functioning of the nervous system, even in higher animals, is possible on the basis of a single neurotransmitter (for review, see ref. [71]). Hence, why do five or six classical neurotransmitters functionally

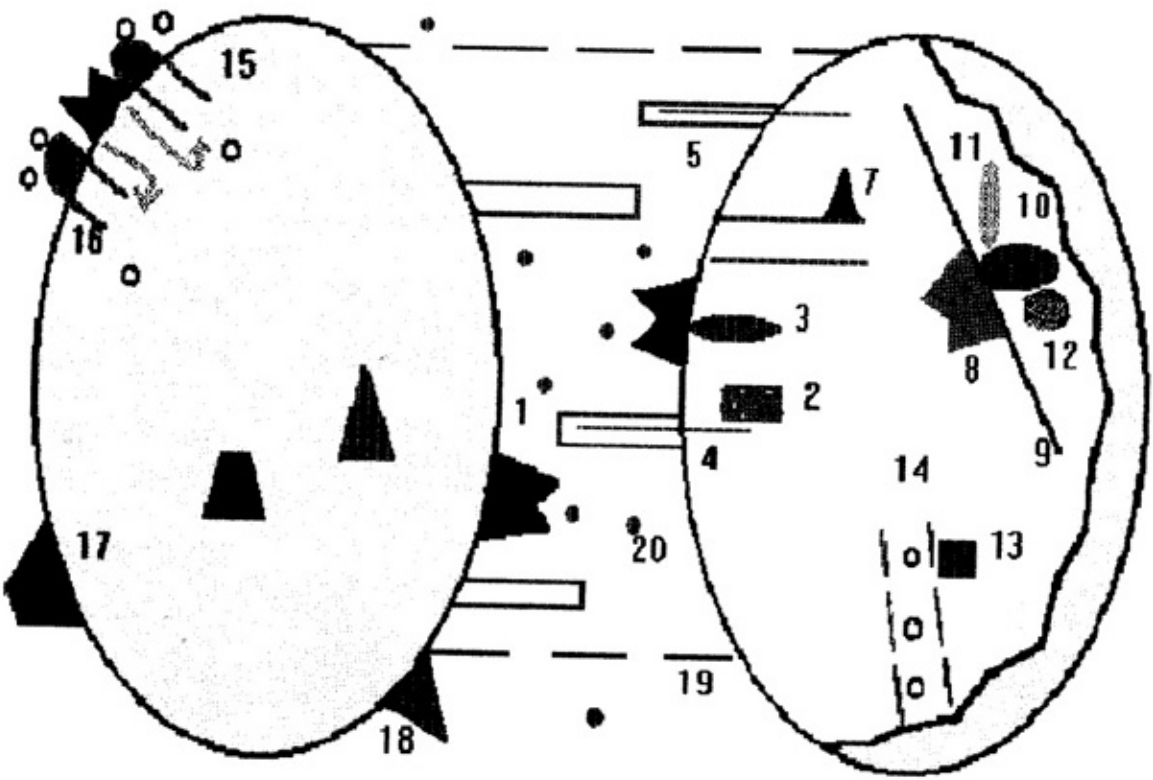


FIGURE 1 Scheme of neurotransmitter mechanisms in 2-blastomere embryo. In left blastomere mainly surface membrane receptors are presented; in right—mainly intracellular receptors and corresponding machinery. In figure:1-surface membrane 5-HT-receptors; 2-intracellular 5-HT-receptors, taking part in blastomere adhesion; 3-adenylyl cyclase in contact membrane; 4-cytoskeletal elements of microvilli; 5-interblastomere space; 6-probable intracellular 5-HT-binding sites on cytoskeletal actin; 7-cytoskeletal elements of cleavage furrow; 8-intracellular 5-HT-receptors, localized on endoplasmic reticulum; 9-endoplasmic reticulum; 10-adenylyl cyclase on endoplasmic reticulum; 11-G_i-protein; 12-G_s-protein; 13-intracellular A-receptor, coupled with cleavage furrow cytoskeletal elements; 14-calcium ions; 15-L-type Ca^{2+}-channels; 16-T-type Ca^{2+}-channels; 17-surface membrane ACh-receptors; 18-probable surface membrane DA-receptors; 19-"zone of protosynapse" (See color plate I)

coupled with second messengers in different ways, actually exist in a single cell, even if the latter is a prospective multicellular organism? However, the term "ostensible" is correct here: all these regulators are necessary. Functions of different prenervous neurotransmitters and, therefore, second messengers coupled with them, coincide only partially (sometimes they are truly antagonistic). For example, suppression of a neurotransmitter function in a one-cell or cleaving embryo severely disturbs or blocks its development, whereas other neurotransmitters can protect embryos only partially, i.e., delay the block of development until a later stage (see above).

In considering this "ostensible redundancy" some interesting and as yet unresolved questions arise. Why are such a variety of multifunctional regulators necessary inside the same cell (zygote or blastomere) and how does this originate? Is it possible to explain the abundance of neurotransmitters and second messengers in adults on the basis of multiplicity of these substances in early embryos? What is the origin and physiological significance of functional coupling between the first and second messengers in the cytoplasm of zygote or blastomere? In other words, how and for what purpose did the system develop that provides for signal generation, transduction, and interaction with an effector inside the same cell?

Some of these questions were discussed above, and here we focus attention on the problem by how multiple and polyfunctional regulatory systems (prospective neurotransmitter systems) developed during evolution. Taking into account data discussed previously, the initial concept may be stated as follows. Phylogenetically, the substances known as neurotransmitters appeared long before the development of the most primitive nervous systems and even multicellular organisms. Neurotransmitter functions repeatedly changed during evolution until the formation of definitive synaptic mechanisms. These evolutionary changes are reflected in the developmental changes of neurotransmitter functions that we observe at the current stage of evolution [1]. Attempts to go further, i.e., to propose an hypothesis concerning the evolutionary origin of neurotransmitters, will unavoidably be speculative.

Scientists supporting one of the hypotheses concerning origin of neurotransmitters proceed from the analysis of synaptic neurotransmission [72] or from data obtained in studies on the regulatory processes in infusoria [73,74]. They believe that neurotransmitters and their receptors initially appeared as the components of a primary mechanism of chemotaxis. The functional significance of this mechanism is to supply the cell with sufficient amounts of substrates for syntheses. Hence, a variety of putative neurotransmitters may be associated with a multiplicity of necessary substrates and, correspondingly, of chemical signals evoking chemotaxis. Coupling of definitive neurotransmitter receptors with the maintenance of ionic homeostasis in the cell (including regulation of activity of ion channels) is derived from electrogenic co-transport of amino acids, similar to that observed during synaptic transmission and in non-excitable cells.

According to the chemotactic hypothesis, neurotransmitter receptors located on the cell surface membrane appeared during evolution earlier than intracellular receptors and are phylogenetic precursors of the latter [73,74]. Intracellular membranes are regarded as derivatives of the outer cell membrane, which enter the cytoplasm by endocytosis together with already formed regulatory-reactive complexes, including neurotransmitter receptors.

An alternative hypothesis suggested by Koshtoyantz [75,76] is based on the sensitivity of protein molecules to a number of endogenous chemical factors. Koshtoyantz suggested that, during evolution, such sensitivity became particularly high and specific in certain proteins (receptor precursors) interacting with certain transformed amino acids (prospective neurotransmitters). He applied this to all classical neurotransmitters. Thus, this hypothesis also assumes evolutionary origin of the multiplicity of neurotransmitters, but regards intracellular neurotransmitter receptors as more ancient than receptors located on the cell surface.

Later, Shmukler and Grigoriev (unpublished) further developed the hypothesis of transformed neurotransmitter precursors. They suggested that transformed amino acids (prospective neurotransmitters)

and the enzymatic system responsible for amino acid transformation initially served as an intracellular probe sensitive to the flow of corresponding amino acids, which are necessary for protein synthesis. If both threshold concentrations triggering this enzymatic system (or corresponding key enzyme in the case of multienzymatic pathway) and sensitivity of proteins (prospective receptors) to transformed amino acid derivatives are sufficiently high, this offers the possibility of control over intracellular levels of protein precursors. In other words, it is easier for a cell to measure small amounts of transformed amino acids interacting with these receptors than the absolute level of regular amino acids. An increase in concentration of a certain neurotransmitter (transformed amino acid) to the threshold level would then indicate that the total amino acid concentration had attained the level sufficient for protein synthesis (Figure 2). According to this hypothesis, the existence of both intracellular and surface membrane receptors for amino acid signal substances is a vestige of two important components of the cell synthetic system

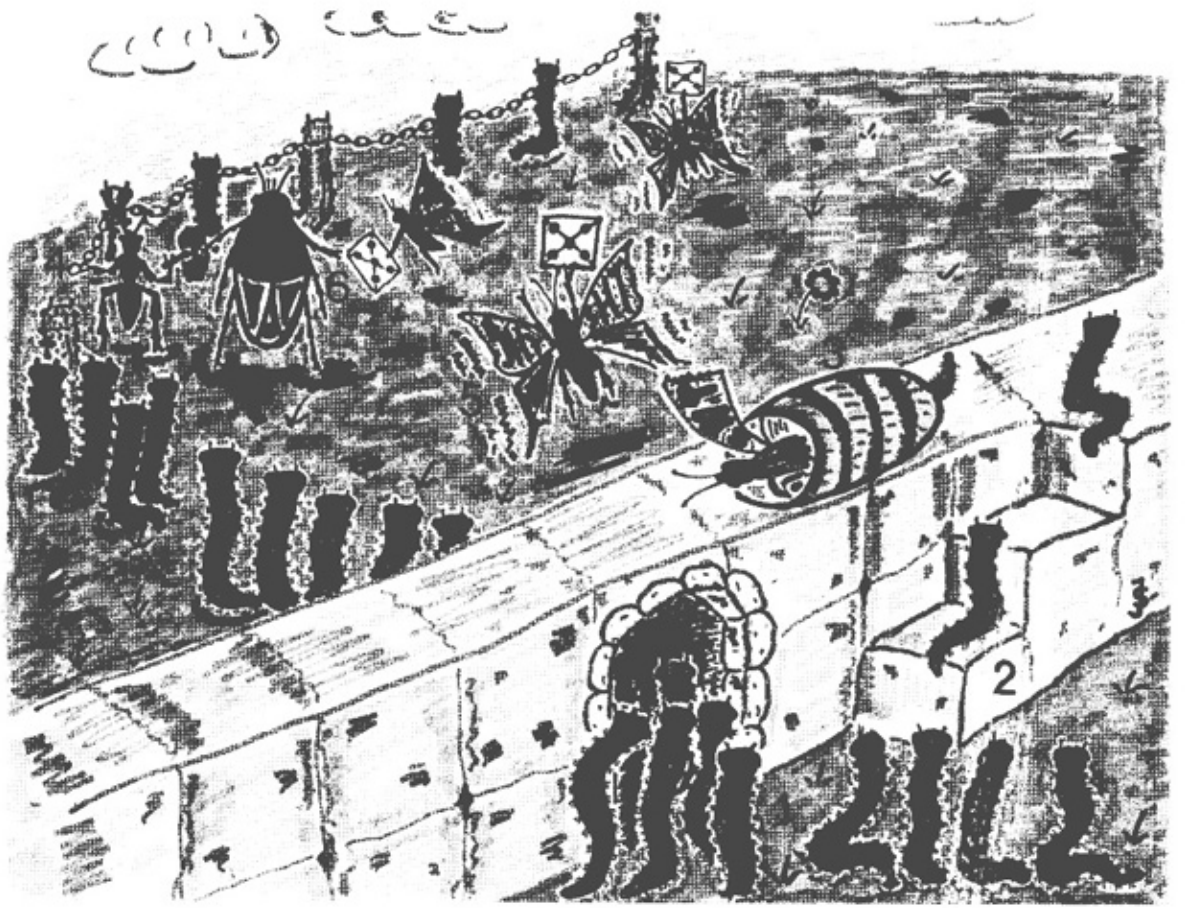


FIGURE 2 Principal scheme for first step of evolutionary neurotransmitter origin (a hypothesis of Kh. S. Koshtoyantz with additions suggested by Yu. B. Shmukler and N. G. Grigoriev).1-Current of amino acid—neurotransmitter presursor; 2-threshold of key neurotransmitter-synthesizing enzyme; 3-neurotransmitter-synthesizing enzymatic system; 4-protein synthesis; 5-newly synthesized neurotransmitter; 6-neurotransmitter receptive structure (See color plate II)

that provide for the flow of substrates from the external medium and control of their intracellular levels.

Both hypotheses assume the genetic relationship of intracellular and membrane neurotransmitter receptors. However, one cannot exclude the possibility that either type of receptor, even for the same neurotransmitter, may have appeared independently.

A scheme combining both hypotheses is also possible (Buznikov, unpublished). According to this scheme, transformed precursors (prospective neurotransmitters) were released from the cell after their interaction with proteins (intracellular receptor precursors) without enzymatic degradation. During subsequent stages of evolution, these ligands induced the appearance of corresponding receptors on the cell surface, as proposed in the chemotactic hypothesis. This scheme favors the idea that intracellular receptors and, consequently, intracellular neurotransmitter functions are phylogenetically older than those associated with the outer cell membrane.

Such a sequence of events takes place during early embryonic development, which supports the combined hypothesis of neurotransmitter system origin. Thus, in early sea urchin embryos, 5-HT-like substances (and, possibly, other classical neurotransmitters) that have interacted with intracellular receptors are released into the medium without enzymatic degradation [2,27]; thereafter, 5-HT functions as an intercellular transmitter at protosynapses, acting via surface membrane receptors [22].

All these hypotheses can be partially verified. Cloning of prenervous intracellular and surface membrane receptors for the same ligand may show their principal similarity or homology. In such a case, the possibility of their independent origin would be less likely. Alternatively, the results of this research may confirm genetic independence of intracellular and surface receptors. Molecular biological methods may also be useful for solving more specific problems concerning interrelations of various groups of prenervous receptors (see above).

Similar logic is applicable to other biosynthetic processes. The transformed precursors of macromolecules other than proteins (in particular, derivatives of fatty acids and nucleotides) can also serve as prospective neurotransmitters and second messengers. This would explain differences in the chemical nature of regulatory substances as well as their multiplicity. As with amino acid derivatives, there are some rare exceptions when a non-transformed precursor becomes a neurotransmitter or second messenger (e.g., glycine, aspartic and glutamic acids, adenosine, or arachidonic acid).

Discussing possible peculiarities of second messenger origin, we should note the following. First, second messengers include various precursors of nucleic acids, lipids, and glycolipids, but no amino acids or their derivatives to which many neurotransmitters belong. Further analysis of this correlation may suggest new approaches to the problems that are beyond the scope of this work at present, in particular, the problem concerning evolutionary linkage of the main biochemical constituents of a living cell. Second, unlike neurotransmitter functions, other functions of second messengers during phylo- and ontogenesis are limited to the intracellular regulatory level. A rare exception to this rule is the morphogenetic role of extracellular cAMP in the slime mold *Dictyostelium discoideum* (see ref. [42]). This situation may reflect the circumstance that nucleotide messengers appeared as products of nuclear metabolism for which the cytoplasm plays a role similar to the extracellular milieu.

A further question is why a variety of regulatory systems and, in particular, coexistence of intracellular and plasma membrane receptors are necessary for early embryogenesis and subsequent stages of development? Generally, the answer is that all this machinery is necessary for maintaining an adequate spatial-temporal organization of regulatory processes throughout ontogenesis, including the adult state [71].

The roles of multiple regulatory systems may be realized in different ways. In adult organisms, factors that ensure appropriate addressing of signals include neurotransmitters and their receptor specificity in neurons, systems of conduction pathways, specific patterns of synapses, spatial isolation of neurotransmitters as intercellular mediators, and second

messengers as intracellular regulators, etc. [71]. All these elements are absent from the one-cell embryos and only appear in cleaving embryos (development of protosynapses, neurotransmitter specificity of blastomeres, etc). The autocrine and semiautocrine systems, based on intricate compartmentalization of neurotransmitters and second messengers, are extremely complex [40,77]. We suggest that such compartmentalization and cellular interactions would be impossible in the absence of coexisting intracellular and membrane neurotransmitter receptors functionally coupled with second messengers. The events described above or in our previous publications [78] illustrate and support this concept. Subsequent studies, even at the current technological level, may provide additional support for this hypothesis.

Spatial-temporal organization of prenervous neurotransmitter processes is a factor ensuring normal embryonic development as well as survival of early embryos at a given moment. Thus, recall switching from intra- to intercellular functioning observed for 5-HT-like substances (and probably other classical neurotransmitters) during each cleavage division. This switching is possible due to coexistence of intracellular and surface membrane neurotransmitter receptors and, correspondingly, to two levels of coupling between first and second messengers. A similar sequence of events, but extended for an indefinite period of time, might take place during phylogenesis. In other words, the development of regulation at both intra- and intercellular levels might be a prerequisite for the appearance of multicellular organisms. Hence, comparative analysis of neurotransmitter systems in protozoans and one-cell metazoan embryos appears to be a feasible approach to an exciting problem concerning the origin of the metazoans and their nervous systems.

In conclusion, we suggest that a multiplicity of neurotransmitter regulatory systems appeared during evolution and became an essential feature of early embryogenesis, which retains its evolutionary significance throughout ontogenesis, while undergoing certain modifications along the way [1]. Corresponding data obtained in studies on early embryos can be very useful for resolving the problem of definitive multiplicity of neurotransmitter and second messenger systems.

Acknowledgements

This work was supported by RFBI grant 93-04-07374 and ISF grants M46000 and M46300.

References

[1] Buznikov G. A., Shmukler Yu. B. and Lauder J. M. (1996). From oocyte to neuron: do neurotransmitters function in the same way throughout development? *Molec. Cell. Neurobiol.*, **16**, 532-559.

[2] Buznikov G. A. (1990). Neurotransmitters in embryogenesis. Chur, Harwood Academic Press. 526 p.

[3] Buznikov G. A. (1967). Low molecular weight regulators in embryonic development. Moscow, Nauka Publishers. 265 p. (in Russian).

[4] Buznikov G. A. and Shmukler Yu. B. (1981). The possible role of "prenervous" neurotransmitters in cellular interactions of early embryogenesis: a hypothesis. *Neurochem. Res.*, **6**, 55-69.

[5] Capasso A., Parisi E., De Prisco P. and De Petrocellis B. (1987). Catecholamine secretion and adenylate cyclase activation in sea urchin eggs. *Cell. Biol. Int. Rep.*, **11**, 457-463.

[6] Emanuelsson H., Carlberg M. and Lowkvist B. (1988). Presence of serotonin in early chick embryos. *Cell Differ.*, **24**, 191-200.

[7] Emanuelsson H. (1992). Autoradiographic localization in polychaete embryos of tritiated mesulergine, a selective antagonist of serotonin receptors that inhibits early polychaete development. *Int. J. Devel. Biol.*, **36**, 293-302.

[8] Kaltner H., Andrae S. and Wittmann J. (1993). Activity of cholinesterases in the japanese quail embryo—effects of dichlorphos on the embryonic development. *Biochem. Pharmacol.*, **45**, 87-92.

[9] Falugi C., Moretti E., Lammerding Koppel M. and Drews U. (1993). In Facchinetti, Henderson, Pierantoni, Polzonetti-Magni (eds), Cellular Communication in Reproduction, Journal of Endocrinology Ltd, Bristol, pp. 101-104.

[10] Buznikov G. A. (1991). The biogenic monoamines as regulators of early (pre-nervous) embryogenesis: new data. In P.S. Timiras, A. Privat, E. Giacobini, J. Lauder (eds), Plasticity and regeneration of the nervous system, Plenum Press, New York and London, pp. 33-48.

[11] Shmukler Yu. B., Grigoriev N. G., Buznikov G. A. and Turpaev T. M. (1986). Regulation of cleavage divisions: participation of prenervous neurotransmitters coupled with second messengers. *Comp. Biochem. Physiol.*, **83C**, 423-427.

[12] Shmukler Yu. B., Grigoriev N. G. and Moskovkin G. N. (1988). Adrenoreceptive structures of early embryos of clawed frog (*Xenopus laevis*) Zhevol. Biokhim. Fiziol., **24**, 621-624.

[13] Small D. H. and Wurtman R. J. (1985). Association of serotonin, dopamine, or noradrenaline with an actin-like component in pheochromocytoma (PC12) cells. *J. Neurochem.*, **45**, 825-831.

[14] Small D. H. and Wurtman R. J. (1985). Binding of 3H-serotonin to skeletal muscle actin. *J. Neurochem.*, **45**, 819-824.

[15] Hardwick J. C. and Parsons R. L. (1995). Requirement of a colchicine-sensitive component of the cytoskeleton for acetylcholine receptor recovery. *Brit. J. Pharmacol.*, **114**, 442-446.

[16] Velez C., Aranega A. E., Fernandez J. E., Melguizo C., Alvarez L. and Aranega A. (1994). Modulation of contractile protein troponin-T in chick myocardial cells by catecholamines during development. *Cell. Molec. Biol.*, **40**, 1189-1199.

[17] Wallace B. G. (1995). Regulation of the interaction of nicotinic acetylcholine receptors with the cytoskeleton by agrin-activated protein tyrosine kinase. *J. Cell Biol.*, **128**, 1121-1129.

[18] Buznikov G. A. and Grigoriev N. G. (1990). The influence of biogenic monoamines and their antagonists on the cortical layer of cytoplasm of early sea urchin embryos. *Zh. evol. biokhim. fisiol.*, **26**, 614-622 (In Russian).

[19] Buznikov G. A. (1984). The action of neurotransmitters and related substances on early embryogenesis. In Papp, J. Gy. (ed.), Developmental Pharmacology, Pergamon Press Ltd, London, pp. 23-59.

[20] Buznikov G. A. and Shmukler Yu. B. (1978). The effect of anti-mediator compounds on intercellular connections in early sea urchin embryos. *Sov. J. Devel. Biol.*, **9**, 141-145.

[21] Buznikov G. A., Mal'chenko L. A., Nikitina L. A., Galanov A. Yu., Pogosyan S. A. and Papayan G. L. (1990). Effect of neurotransmitters and their antagonists on oocyte maturation. 2. Effect of serotonin antagonists on the sensitivity of starfish oocytes to forskolin and ionomycin. *Sov. J. Devel. Biol.*, **21**, 431-436.

[22] Shmukler Yu. B. (1993). Possibility of membrane reception of neurotransmitter in sea urchin early embryos. *Comp. Biochem. Physiol.*, **106C**, 269-273.

[23] Baccetti B. B., Burrini G. A., Collodel G., Moretti E., Piombomi P., Falugi C., Trielli F., Lammerding-Koppel M. and Drews U. (1995). Cholinergic receptors localization and possible role in Paracentrotus lividus fertilization and block to polyspermy. In: *Abs. Congress Eur. Developm. Biol. Organisation*, p. 131. Toulouse, July 9-13, 1995.

[24] Falugi C. (1993). Localization and possible role of molecules associated with the cholinergic system during "non-nervous" developmental events. *Eur. J. Histochem.*, **37**, 287-294.

[25] Falugi C. and Prestipino G. (1989). Localization of putative nicotinic cholinoreceptors in the early development of Paracentrotus lividus. *Cell. Molec. Biol.*, **35**, 147-161.

[26] Shmukler Yu. B. (1981). Cellular interactions in early sea urchin embryos. III. The effect of neuropharmacological compounds on division type of Scaphechinus mirabilis half embryos. *Sov. J. Dev. Biol.*, **12**, 263-267.

[27] Markova L. N., Buznikov G. A., Kovacevic N., Rakic L., Salimova N. B. and Volina E. V. (1985). Histochemical study of biogenic monoamines in early (prenervous) and late embryos of sea urchins. *Int. J. Devel. Neurosci.*, **3**, 493-500.

[28] Shmukler Yu. B., Buznikov G. A. and Whitaker M. J. (1995). The effect of serotonergic substances on free calcium ion concentrations in the *Lytechinus pictus* embryos. In: *Abs. Congress Eur. Developm. Biol. Organisation*, p. 64. Toulouse, July 9-13, 1995.

[29] Capasso A., Creti P., De Petrocellis B., De Prisco P. and Parisi E. (1988). Role of dopamine and indolamine derivatives in the regulation of sea urchin adenylate cyclase. *Biochem. Biophys. Res. Comm.*, **154**, 758-764.

[30] Carginale V., Capasso A., Madonna L., Borelli L. and Parisi E. (1992). Adenylate cyclase from sea urchin eggs is positively and negatively regulated by D-1 and D-2 Dopamine receptors. *Exp. Cell Res.*, **203**, 491-494.

[31] Lauder J. M. (1993). Neurotransmitters as growth regulatory signals: role of receptors and second messengers. *Trends Neurosci.*, **16**, 233-240.

[32] Shilling F. M., Carroll D. J., Muslin A. J., Escobedo J. A., Williams L. T. and Jaffe L. A. (1994). Evidence for both tyrosine kinase and G-protein-coupled pathways leading to starfish egg activation. *Devel. Biol.*, **162**, 590-599.

[33] Berridge M. J. and Dupont G. (1994). Spatial and temporal signalling by calcium. *Curr. Opin. Cell Biol.*, **6**, 267-274.

[34] Berridge M. J. (1995). Calcium signalling and cell proliferation. *Bioessays*, **17**, 491-500.

[35] Jaffe L. F. (1995). Calcium waves and development. In Bock, G. R.; Ackrill, K. (eds), Calcium Waves, Gradients and Oscillations, John Wiley & Sons Ltd, London, pp. 4-12.

[36] Rostomyan M. A., Abramian K. S., Buznikov G. A. and Gusareva E. V. (1985). Electronmicroscopy cytochemical detection of adenylate cyclase in early sea urchin embryos. *Tsitologiya*, **27**, 877-881 (in Russian).

[37] Stanley-Samuelson D. W. (1994). The biological significance of prostaglandins and related eicosanoids in invertebrates. *Amer. Zoologist*, **34**, 589-598.

[38] Ciapa B., Pesando D., Wilding M. and Whitaker M. (1994). Cell-cycle calcium transients driven by cyclic changes in inositol trisphosphate levels. *Nature*, **368**, 875-878.

[39] Gillot I. and Whitaker M. (1994). Calcium signals in and around the nucleus in sea urchin eggs. *Cell Calcium*, **16**, 269-278.

[40] Sardet C., McDougall A. and Houliston E. (1994). Cytoplasmic domains in eggs. *Trends Cell Biol.*, **4**, 166-72.

[41] Silver R. B., Oblak J. B., Jeun G. S., Sung J. J. and Dutta T. C. (1994). Leukotriene B-4, an arachidonic acid metabolite, regulates intracellular free calcium release in eggs and mitotic cells of the sand dollar *(Echinaracnius parma)*. *Biol. Bull.*, **187**, 242-244.

[42] McMahon D. (1974). Chemical messengers in development: a hypothesis. *Science*, **185**, 1012-1021.

[43] Stith B. J., Goalstone M., Silva S. and Jaynes C. (1993). Inositol 1,4,5-trisphosphate mass changes from fertilization through 1st-cleavage in *Xenopus laevis*. *Molec. Biol. Cell*, **4**, 435-443.

[44] Berridge M. J. (1975). Control of cell division: an unifying hypothesis. *J. Cycl. Nucl. Res.*, **1**, 305-320.

[45] Berridge M. J. (1984). Cellular control through interactions between cyclic nucleotides and calcium. *Adv. Cycl. Nucl. Protein Phosphoryl. Res.*, **17**, 329-336.

[46] Yasumasu I., Hino A. and Asami K. (1977). Effect of dibutyryl adenosine 3′,5′-cyclic monophosphate on several metabolic systems in sea urchin eggs. *Cell Struct. Funct.*, **2**, 11-20.

[47] Ishida K. and Yasumasu I. (1982). The periodic changes in adenosine 3′,5′-monophosphate concentration in sea urchin eggs. *Biochim. Biophys. Acta*, **720**, 266-273.

[48] Sadokova I. E. (1982). The dynamics of cyclic nucleotide content in developing embryos of sea urchin *Strongylocentrotus intermedius*. *Sov. J. Dev. Biol.*, **13**, 435-440.

[49] Shmukler Yu. B. and Grigoriev N. G. (1984). Cellular interactions in early sea urchin embryos. V. New data related to control mechanisms of micromere formation. *Sov. J. Dev. Biol.*, **15**, 308-310.

[50] Renaud F., Parisi E., Capasso A. and De Prisco E. P. (1983). On the role of serotonin and 5-methoxytryptamine in the

regulation of cell division in sea urchin eggs. *Develop. Biol.*, **98**, 37-47.

[51] Carginale V., Borrelli L., Capasso A. and Parisi E. (1995). Changes in dopamine uptake and developmental effects of dopamine receptor inactivation in the sea urchin. *Molec. Reprod. Develop.*, **40**, 379-385.

[52] Buznikov G. A., Martynova L. E., Marshak T. L., Galanov A. Yu., Dungenova R. E., Nikitina L. A. and Mileusnic R., Rakic L. (1993). The effect of protein kinase C activators and inhibitors MA on early echinoderm embryos. *Russian J. Dev. Biol.*, **24**, 172-181.

[53] Yazaki I., Tosti E. and Dale B. (1995). Cytoskeletal elements link calcium channel activity and the cell cycle in early sea urchin embryos. *Development*, **121**, 1827-1831.

[54] Dale B., Talevi R. and De Felice L. J. (1991). L-type Ca^{2+} currents in ascidian eggs. *Exptl Cell Res.*, **192** (1): 302-306.

[55] Arnoult C. and Villaz M. (1994). Differential developmental fates of the two calcium currents in early embryos of the ascidian *Ciona intestinalis*. *J. Membr. Biol.*, **137**, 127-135.

[56] Miyazaki S., Katayama Y. and Swann K. (1990). Synergistic activation by serotonin and GTP analogue and inhibition by phorbol ester of cyclic Ca^{2+} rises in hamster eggs. *J. Physiol. (L.)*, **426**, 209-227.

[57] Allbritton N. L., Meyer T. and Stryer L. (1992). Range of messenger action of calcium ion and inositol 1,4,5 triphosphate. *Science*, **258**, 1812-1815.

[58] Rasmussen E. and Barrett P. (1984). Calcium messenger system: an integrated view. *Physiol. Rev.*, **61**, 938-984.

[59] Shmukler Yu. B., Grigoriev N. G. and Martynova L. E. (1987). Changes of cell surface of *Xenopus laevis* blastomeres after cAMP and calcium ions microinjection. *Dokl. AN SSSR*, **294**, 507-510 (in Russian).

[60] Conrad G. W. and Davis S. E. (1977). Microiontophoreticinjection of calcium ions or cyclic AMP causes rapid shape changes in fertilized eggs of *Ilyanassa obsoleta*. *Devel. Biol.*, **61**, 184-201.

[61] Shen S. S. and Ricke L. A. (1989). Protein kinase C from sea urchin eggs. *Comp. Biochem. Physiol.*, **92B**, 251-254.

[62] Nelsestuen G. L. and Bazzi M. D. (1991). Activation and regulation of protein kinase C enzymes. *J. Bioenerg. Biomembr.*, **23**, 43-62.

[63] Berridge M. J. (1993). Intracellular channels and calcium spiking. *Biomed. Res.*, **14**(Suppl. 2): 21-27.

[64] Gallo C. J., Hand A. R., Jones T. L. Z. and Jaffe L. A. (1995). Stimulation of *Xenopus* oocyte maturation by inhibition of the G-protein alpha(s) subunit, a component of the plasma membrane and yolk platelet membranes. *J. Cell Biol.*, **130**, 275-284.

[65] Bustamante J. O., Liepins A. and Hanover J. A. (1994). Nuclear pore complex ion channels. *Molec. Membr. Biol.*, **11**, 141-150.

[66] Dale B., Defelice L. J., Kyozuka K., Santella L. and Tosti E. (1994). Voltage clamp of the nuclear envelope. *Proc. Roy. Soc. London Ser. B*, **255**, 119-124.

[67] McEnery M. W., Snowman A. M. and Snyder S. H. (1994). The association of endogenous G omicron alpha with the purified omega-conotoxin GVIA receptor. *J. Biol. Chem.*, **269**, 5-8.

[68] Kinnally K. W., Zorov D. B., Antonenko Y. N., Snyder S. H., McEnery M. W. and Tedeschi H. (1993). Mitochondrial benzodiazepine receptor linked to inner membrane ion channels by nanomolar actions of ligands. *Proc. Natl Acad. Sci. USA*, **90**, 1374-1378.

[69] Sardet C., Gillot I., Rusher A., Payan P., Girard J. P. and Derenzis G. (1992). Ryanodine activates sea urchin eggs. *Develop. Growth Differ.*, **34**, 37-42.

[70] Lindsay A. and Williams A. J. (1994). How does ryanodine modify ion handling in the sheep cardiac sarcoplasmic reticulum Ca^{2+}-release channel? *J. Gen. Physiol.*, **104**, 425-447.

[71] Sakharov D. A. (1990). Neurotransmitter diversity: functional significance. *Zh. evol. Biokhim. Fisiol.*, **26**, 734-741 (in Russian).

[72] Boyd C. A. R. (1979) Chemical neurotransmission: an hypothesis concerning the evolution of neurotransmitters substances. *J. Theoret. Biol.*, **76**, 415-417.

[73] Csaba G. (1981). Ontogeny and phylogeny of hormone receptors. Basel, Karger, 172 p.

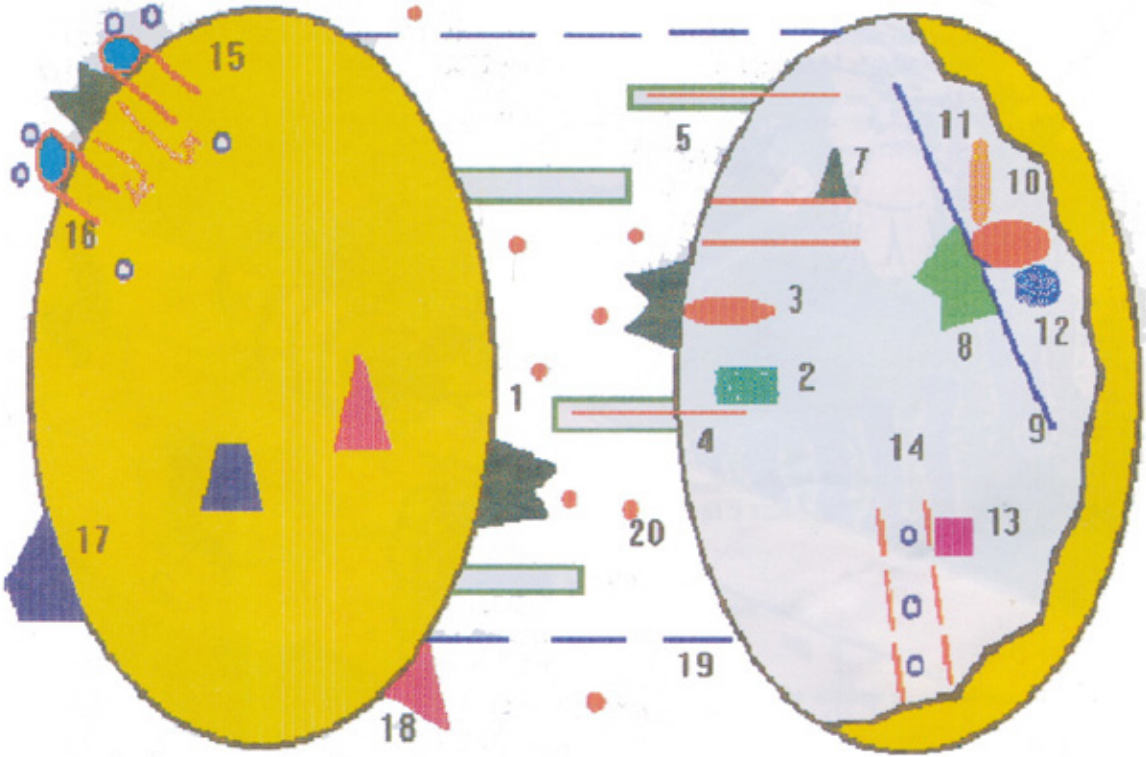
[74] Csaba G. (1984). The present state in the phylogeny and ontogeny of hormone receptors. *Hormone Metabol. Res.*, **16**, 329-335.

[75] Koshtoyantz Kh. S. (1951) Proteins, Metabolism, and Neural Regulation, Moscow, *Publ. House USSR Acad. Sci.*, pp. 100 (in Russian).

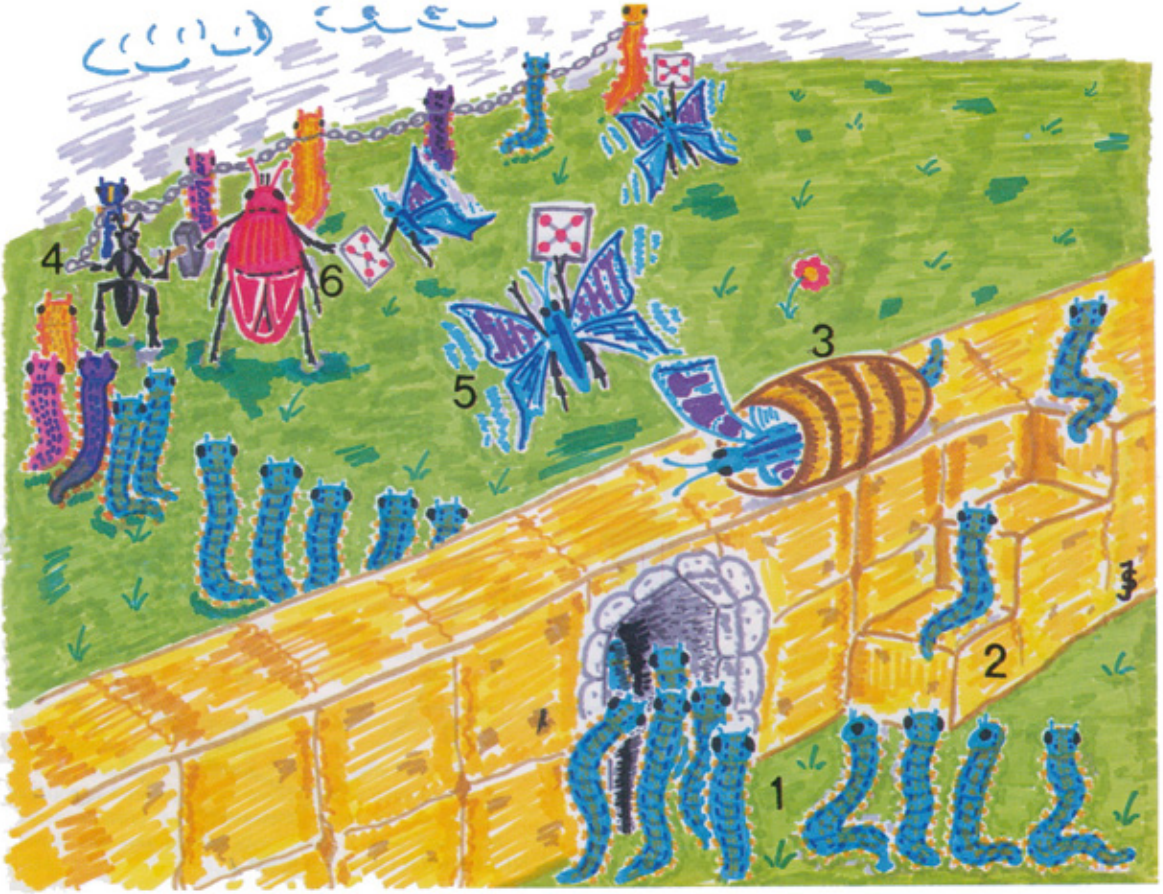
[76] Koshtoyantz Kh. S. (1963). The Problems Related to Enzymochemistry of Excitation and Inhibition Events, and to Evolution of Nervous System Function, Moscow, Publ. House USSR *Acad. Sci.*, pp. 31 (in Russian).

[77] Fryxell K. J. (1995). The evolutionary divergence of neurotransmitter receptors and second-messenger pathways. *J. Molec. Evolution*, **41**, 85-97.

[78] Buznikov G. A., Manukhin B. N., Rakic L., Kudryashova N. I., Mdzhoyan O. L., Ovsepyan T. R. and Khromov-Borisov N. V. (1982). Sensitivity gradient to antitransmitter drugs of normal and stratified *Arbacia lixula* embryos. *Sov. J. Develop. Biol.*, **13**, 309-313.



PERSPECTIVES ON DEVELOPMENTAL NEUROBIOLOGY, Vol 5, No 4
COLOUR PLATE I. See Yuri B. Shmukier and Gennady A. Buznikov



PERSPECTIVES ON DEVELOPMENTAL NEUROBIOLOGY, Vol 5, No 4
COLOUR PLATE II. See Yuri B. Shmukier and Gennady A. Buznikov