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Comparative studies on the roles of calcium ions and gap Junctions in the regulation of secretory and contractile processes: Biochemical and pharmacologic approaches

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Abstract

This article presents a review of our own studies on the role of calcium ions and gap junctions in the regulation of prolactin and growth hormone secretion by rat adenohypophyseal cells in primary cultures. The article reviews also our own data on interactions of calcium ions with functional activity of gap junctions in the regulation of contractions of isolated rat uterus. The discussion is presented of literature evidence on the role of gap junctions in the regulation of insulin secretion by pancreatic islets and contractions of vascular smooth muscle tissues, as well as on the perspectives of studying the contribution of gap junctional intercellular communication in the framework of DOHaD (Developmental Origins of Health and Disease). Finally the article considers briefly the elaboration and possible use of pharmacologic agents modulating functional activity of gap junctions in the treatment of several diseases.

Introduction

This article has rather long history lasting for almost 40 years. In fact, already in the eighties and nineties of the last century we studied the roles of calcium ions (Ca^{2+}) and gap junctions in the regulation of prolactin (PRL) and growth hormone (GH) secretion by rat adenohypophyseal cells in primary cultures [1-8]. Somewhat later we applied similar approaches for studying the roles of calcium ions and gap junctions in the regulation of contractile activity of isolated rat uterus [9,10]. Although many years have passed from that period, these data have not lost their significance and in addition, practically nobody has tried to perform comparative analysis of the roles of calcium ions and gap junctions in the regulation of secretory and contractile processes. We have decided to fill this gap, reviewing our own data on PRL and GH secretion by adenohypophyseal cells and our results on contractile activity of isolated rat uterus. Finally, we shall discuss literature evidence on the role of gap junctions in regulation of insulin secretion by beta-cells of pancreatic islets and contractile activity of vascular smooth muscle tissues, in order to compare our own data with bibliographic evidence obtained on other experimental models, evaluating other secretory and contractile processes.

The role of calcium ions and gap junctions in the regulation of prolactin and growth hormone secretion

First of all, our experiments performed on primary cultures of rat adenohypophyseal cells have shown that removal of calcium ions from the incubation medium resulted in the decrease of basal PRL secretion, but did not change basal GH release. However, in the medium without calcium ions, the stimulatory influence of depolarizing concentrations of potassium (K^+) ions (30 or 55 mM) on both PRL and GH release was completely prevented [1,2]. We have established also that ions of cobalt (Co^{2+}), the antagonist of calcium ions, inhibited PRL secretion, whereas ions of barium (Ba^{2+}), considered to be an agonist of calcium ions, stimulated PRL and GH release, and in the medium without calcium ions these effects of Ba^{2+} were greatly enhanced [2,3]. Finally, we have observed that in the medium without calcium ions, the stimulatory action of thyroliberin (TRH) on PRL secretion was decreased, whereas, in contrast, it was necessary to add the excess of calcium ions (5 mM) to incubation medium, in order to prevent the inhibitory influence of somatostatin on PRL and GH release [4,5].

Already in the decade of nineties of the last century we have observed in the experiments on primary cultures of rat adenohypophyseal cells that octanol, a pharmacologic agent blocking gap junctions, did not influence basal PRL secretion, but inhibited PRL release stimulated by TRH or dibutyryl-derivative of cyclic AMP (DbcAMP) [6-8]. In addition, octanol did not influence basal GH secretion, as well as GH release stimulated by dibutyryl-derivative of cyclic GMP, but this agent inhibited GH release stimulated by TRH or DbcAMP [7,8].

In conclusion, we can affirm that calcium ions and gap junctions play very important and quite selective roles in the regulation of secretory processes in anterior pituitary.

Interrelated roles of calcium ions and gap junctions in the regulation of contractile activity of isolated rat uterus

In the experiments on adult rat uterus [9,10], we have established that in the incubation medium with 1-2 mM Ca^{2+}, gap junction-blocking agent octanol, already in the concentration of 0.3 mM,

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inhibited uterine contractions induced by oxytocin, however in the medium with excess Ca^{2+} (5 mM) or in the cases of elevated oxytocin concentrations, higher level of octanol (0.6 mM) was necessary for inhibition of oxytocin-induced uterine contractile activity.

Similar situation was observed in relation to acetylcholine (ACh), i.e. with the increase of concentration of this neurotransmitter, parallel augment of octanol level was necessary for inhibiting the contractions of adult rat uterus. On the other hand, in the case of intermediate ACh concentration, it was possible to prevent inhibitory octanol action by means of addition of Ca^{2+} excess to incubation medium. Similar peculiarities were observed also in the experiments on the uterus of adult rats with depolarizing concentration of potassium ions (29mM), i.e. the addition of Ca^{2+} excess to incubation medium decreased the inhibitory octanol action on stimulated uterine contractions.

In the experiments on the uterus of adult rats we have shown the inhibitory influence of verapamyl, the antagonist of calcium channels, on uterine contractions induced by depolarizing concentration of potassium ions (29 mM) or by ACh. We have performed also the comparison of inhibitory actions of verapamyl, octanol and adrenaline on uterine contractions induced by ACh or Ba^{2+} (1 mM). Moreover, it was demonstrated that the addition of Ca^{2+} excess to incubation medium dose-dependently blocked the actions of all three inhibitors of contractions of adult rat uterus (verapamyl, octanol and adrenaline).

Several experiments were performed on isolated uterus of prepubertal rats. In particular, it was found that addition of Ca^{2+} excess to incubation medium was able to partially counteract inhibitory octanol influence on uterine contractions caused by depolarizing concentration of potassium ions (54 mM). Similar situation was observed in experiments on the uterus of prepubertal animals also in the case of uterine contractions induced by Ba^{2+}.

Therefore, summarizing the facts described above, we can conclude that octanol, i.e. the agent blocking gap junctions, dose-dependently inhibited contractile activity of isolated uterus of adult rats, whereas Ca^{2+} excess in the incubation medium dose-dependently prevented this inhibitory octanol action, i.e. the action of octanol combined with Ca^{2+} is similar to the effects of other inhibitors of uterine contractions, verapamyl and adrenaline, combined with Ca^{2+} . In addition, the interaction of octanol and Ca^{2+} excess was partially demonstrated also on isolated uterus of prepubertal animals.

The role of gap junctions in regulation of other secretory and contractile processes

The literature search was obviously realized before performing our experiments in the decades of eighties and nineties of the last century. Moreover, this bibliographic search was performed recently once more. In present article we shall limit the discussion by describing literature evidence on insulin secretion and contractile activity of vascular smooth muscle tissues.

The most important contribution to studies on the role of gap junctions in the regulation of insulin secretion was made by Swiss research group of Paolo Meda [11]. These investigators have shown, in particular, that heptanol, i.e. an agent similar to octanol in its capacity of blocking the gap junctions, inhibited insulin secretion by pancreatic islets, stimulated by high glucose concentrations, and this effect was totally reversible after removal of heptanol from incubation medium. It is interesting that heptanol did not cause any influence on insulin secretion by isolated, single beta-cells of pancreatic islets, i.e. for realization of inhibitory heptanol action, the presence of intercellular gap junctions was absolutely necessary.

On the other hand, inhibitory heptanol and octanol influence on contractile activity of smooth muscle tissues was shown in experiments on isolated aorta or mesenteric vascular preparations [12-14], however none of these works attempted to study combined effects of gap junction blockers and Ca^{2+} excess in the incubation medium.

General discussion of our own results and literature data

One of the most active authors in studying the gap junctions, James E. Trosko has written recently in his review article that unfortunately, during the last decades biomedical researchers in the whole world largely ignored this important theme [15]. We completely agree with this critical notion, confirming for example, the scarcity of investigations on the role of gap junctions in the regulation of contractile uterine activity after a series of electron-microscopic studies of Canadian group of R.E. Garfield in the eighties of the last century [16] and after our results on effects of octanol, although already in 1996 we were able to attract the attention to them of Juan Saez, well-known researcher of gap junctions during Pan-American Congress of Biochemistry and Molecular Biology in Chile.

Nevertheless, certain progress in understanding the role of gap junctions in regulation of secretory and contractile processes was achieved in general during the last decades. In fact, soon after publication of our works on PRL and GH secretion, it was established that in rat anterior pituitary in vivo and in primary culture of adenohypophyseal cells, the gap junctions were revealed, first of all, between so called folliculo-stellate cells and lactotrophs or somatotrophs, i.e. the cells secreting PRL and GH respectively [17]. By the way, significant participation of folliculo-stellate cells and intercellular communication mediated by gap junctions are forcing us to introduce certain corrections to our theoretical model of anterior pituitary cytoarchitectonics on the basis of cell flows [18,19].

In addition, the advances in studying the connexins, i.e. proteins representing the principal component of gap junctions, have resulted in the identification of important differences between endocrine glands containing the connexin with calculated molecular mass of 43 kilodalton (Cx43) and exocrine glands containing Cx32 [20]. Quite interesting aspect consists of the evidence that agents activating exocrine secretion of amylase and other enzymes by acinar pancreatic cells, do not stimulate, but really inhibit functional activity of their gap junctions, whereas heptanol, i.e. the blocker of gap junctions, stimulates exocrine activity of acinar pancreatic cells.

Although, on our opinion, it is too early to make significant conclusions on this basis, we consider to be quite appropriate in offering preliminary suggestion that such difference in the contribution of gap junctions to regulation of endocrine and exocrine secretion may be partially related to the necessity of higher stimulation of at least some endocrine glands during the phase of active functionality of the organism (or stress) and on the contrary, higher stimulation of exocrine secretion in the phase of relative rest of the body that in turn, depends on the balance of activities of sympathetic and parasympathetic parts of autonomic / vegetative nervous system.

Although in the eighties of the last century we could not study the role of gap junctions in the regulation of functional activities of cultured rat liver cells by means of evaluating the effects of octanol, nevertheless it continues to be not clarified, why DbcAMP in certain doses and incubation durations did not stimulate, but really inhibited both the

secretion of serum albumin and total RNA and protein biosynthetic rates in cultured hepatocytes [21,22]. It is possible that partially the answer to this question can be given, considering that in accord with its secretory function, the liver is much more similar to exocrine glands, since this organ is secreting bile to the lumen of intestinal tract, whereas the secretion of albumin and other blood serum proteins by hepatocytes is radically different in its mechanisms from the release of e.g., pituitary hormonal proteins.

Conclusion

Already in 1990 we have tried to attract the attention to gap junction theme, suggesting the possible participation of so called intercrine, or intercellular signaling communication in the regulation of various physiologic processes [23]. In fact, at that time and until present moment the researchers usually considered to greater extent the extracellular and intracellular types of signaling communication, where the principal role are played respectively by hormones and other bioactive substances transported by blood and other biological fluids and by so called secondary messengers of hormonal signals, destined for acting inside the cells (cyclic nucleotides, calcium ions, etc.) [15].

Nevertheless, it was shown already in the experiments on paired cardiomyocytes that cAMP and angiotensin II generated inside the cells, are able to participate in the transfer of regulatory signals through gap junctions to neighbor cells [24,25]. In addition, the oscillations of intracellular Ca^{2+} concentration can be propagated along the tissues and in cell cultures by means of gap junctions [26], in spite of the fact that in high concentrations ($> 10^{-5}$ M) the same calcium ions are able to shut down the gap junctions. Perhaps, exactly the last fact found already in the sixties of the last century [27], has delayed to certain extent the studies of interactions between calcium ions and gap junctions in the regulation of physiologic processes, although already in the original works of the sixties and later on it was frequently noted that such influence of high intracellular Ca^{2+} concentrations is necessary for preventing the transfer of lesions from cells with disrupted integrity of cytoplasmic biomembrane to the neighbor cells.

Therefore, summarizing all described above, it is possible to make a conclusion that there exists really essential similarity in the contribution of calcium ions and gap junctions to the regulation of at least some secretory and contractile processes. Many authors explain such similarity in the following manner: for attaining sufficient intensity of hormone release to the blood or contraction of smooth muscle tissues, it is necessary to achieve the combined and synchronized activation of functional activity of separate cells interconnected by means of gap junctions between them [28]. It is important also that gap junctions are necessary probably for enhancing the sensitivity of endocrine organs and smooth muscle tissues to natural bioregulators and pharmacologic agents [12]. It means that together with calcium ions, intercellular communication by means of gap junctions plays very important roles in regulation of various secretory and contractile processes.

The question about changes of intercellular communication mediated by gap junctions in the ontogeny remains poorly explored. Somewhat recently James E. Trosko [15] tried to find the connection between the phenomena of programming / imprinting in the framework of DOHaD concept and alterations of gap junctions during cell differentiation, but in general, this important question remains completely open for future studies. Earlier we have investigated possible differences in the role of calcium ions and cyclic nucleotides in cultured anterior pituitary cells of rats of different age groups [29], and in experiments on isolated rat uterus we have made an attempt of comparing the role of gap junctions in regulation of contractile uterine activity, depending on the extent of maturity of this organ (see above). Without any doubt, these interesting studies should be continued. Especially important seems to be the question about participation of serotonin and other compounds with low molecular weight, capable to penetrate through gap junctions, in embryogenesis [30].

In conclusion, we would like to note that there is significant, but still latent potential in elaborating new pharmacologic agents influencing gap junctions [31]. This notion is confirmed by experimental works evidencing the potential capacity of heptanol or octanol to limit cellular lesions in ischemic heart disease and epilepsy [32,33]. There is the necessity of more intense investigations on the role of gap junctions in heart failure [34] and carcinogenesis [15]. Therefore, on our opinion, the biochemists, pharmacologists, and other investigators in biomedical sciences cannot ignore, no more, the gap junction theme.

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