EXPERIMENTAL BIOLOGY

Possible Involvement of Gap Junctions in Realizing Effects of Stimulators of Secretory Processes in the Pituitary

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The important role of adenosine 3'5'-cyclic monophosphate (cAMP) in the regulation of a variety of metabolic processes, and proliferation and differentiation of cells is now beyond question. Synthesized in the near-membrane area from ATP due to the action of adenylate cyclase, cAMP then binds with the regulatory subunit of cAMP-dependent protein kinase, thereby resulting in the release of the catalytic subunit, which phosphorylyzes the proper protein substrates, while cyclic-nucleotide phosphodiesterase degrades unbound cAMP [9]. However, while this harmonious scheme is fitting for a general, abstract cell, it ignores the heterogenicity and cell-to-cell interaction in existing actual tissue cell populations. Experiments both *in vivo* [14] and *in vitro* with primary confluent cultures [10] showed that pituitary cells possess intercellular microchannels, so-called gap junctions, which are thought to carry out the transport of low-molecular subtances (m.w.<800-1000 D). These gap junctions represent a special class of ultrastructures, which differ from ionic channels in their properties [7].

Earlier, we used octanol, an agent interfering with cell-to-cell communication, for evaluation of the role of gap junctions in the regulatory influence of cAMP on cultured pituitary cells of prepubertant rats [4]. It was established that octanol does not affect basal prolactin secretion, while it blocks the stimulatory effect of $N^6,O^{2'}$-dibutyryl cAMP (DbcAMP). Analogous results on the effect of a similar agent, heptanol, were obtained on isolated pancreatic islets with respect to stimulation of insulin secretion by agents inducing the accumulation of cAMP in the cells [12]. These investigations suggested that gap junctions play an important role in intercellular communications and in the generalization of the responses of functional cell assemblages to hormonal bioregulators.

Here we present the results of further investigation of the effect of octanol on secretory processes. For more comprehensive analysis we used cultured pituitary cells obtained from animals of another age group. Not only lactotrophic but also other cell elements, somatotrophic cells, served as an object of the investigation. The set of regulators of secretion was also extended and dibutyryl cyclic guanosine 3'5'-monophosphate (DBcGMP) and thyroliberin (thyrotropin releasing hormone; TRH) were used in addition to DBcAMP.

MATERIALS AND METHODS

Neonatal (5-7-day-old) rats of both sexes were taken for the experiments. The methods of prepa-

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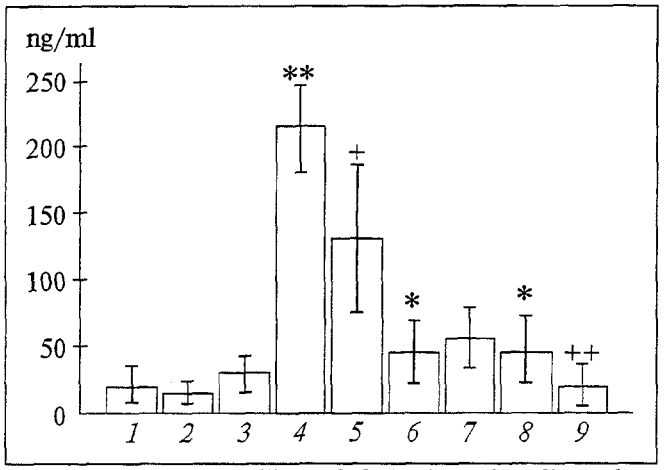

Fig. 1. Interaction of dibutyryl derivatives of cyclic nucleotides (2.5 mM) and TRH (40 ng/ml) with octanol in regulation of GH release from cultured pituitary cells of neonatal rats. Ordinate: concentration of GH in the medium, ng/ml. *1*) control; *2*) octanol; *3*) sodium butyrate (2.5 mM); *4*) DbcAMP; *5*) DbcAMP+octanol; *6*) DbcGMP; *7*) DbcGMP+octanol; *8*) TRH; *9*) TRH+octanol. $n=5-9$ per group. *: $p<0.01$ and **: $p<0.001$ in comparison with the control; +: $p<0.02$ in comparison with DbcAMP; ++: $p<0.01$ in comparison with TRH.

ration and cultivation of the primary pituitary cell culture [6] with modifications [5] and homologous radioimmunoassay of growth hormone (GH) [1] and prolactin [2] were described earlier. After 5 days of cultivation the cells were incubated during 3 hours with the tested agents in medium 199 containing 2 mg/ml bovine serum albumin. The following preparations were used: TRH (Serva, Germany), DbcAMP and DbcGMP (Sigma, USA), and octanol and butyrate of domestic manufacture. The results are presented as $M\pm m$; the reliability of differences was evaluated using the Student t test.

RESULTS

The data presented in Fig. 1 show that octanol and butyrate did not change the basal secretion of GH in cultured pituitary cells of neonatal rats, while DbcAMP, DbcGMP, and TRH elevated GH secretion 10.8, 2.1, and 2 times, respectively. The fact that butyrate did not affect GH secretion suggests that butyrate, which may be released during hydrolysis of these compounds in the course of incubation, is not responsible for the above effects of dibutyryl derivatives of cyclic nucleotides. Octanol did not change the secretogenic effect of DbcGMP, but it inhibited the stimulatory effects of DbcAMP and TRH on GH secretion.

TABLE 1. Interaction of TRH (40 ng/ml) and Octanol (0.6 mM) in Regulation of Prolactin Release from Cultured Pituitary Cells of Neonatal Rats ($M\pm m$)

Group	Concentration of prolactin im medium, ng/ml	p
Control	40.5±9.7 (8)	–
TRH	77.7±8.1 (6)	$p_{1-2}<0.01$
Octanol	24.3±6.4 (5)	$p_{1-3}>0.05$
TRH+octanol	35.6±10.7 (5)	$p_{2-4}<0.02$
		$p_{3-4}>0.05$

Note. In parentheses is the number of observations per group.

As is shown in Table 1, octanol did not reliably change basal prolactin secretion, but it blocked the secretogenic effect of TRH on lactotrophic cells.

Thus, using another age group and another pituitary hormone, we succeeded in confirming our previous data concerning an inhibitory effect of octanol on cAMP analog-induced secretory processes in the pituitary [4]. At the same time, no effect of octanol was found on the release of pituitary hormone induced by cGMP analog.

Other authorities have shown an important role of cAMP, but not cGMP, in the regulation of gap junction-mediated cell-to-cell communication [13]. The difference in the regulatory contribution of cyclic nucleotides corroborated here deserves special investigation.

As for the octanol-induced blockade of the secretogenic effect of TRH, futher study is required for elucidating the role of particular components of the second messenger system (cyclic nucleotides, Ca^{2+}-calmodulin, protein kinase C, etc. [9]) in the mechanism of this phenomenon. Unlike somatoliberin, the effect of TRH on secretion of HG in adenopituitary cells was shown [15] not to be mediated through cAMP. However, the participation of various messengers in the secretogenic effect of TRH on both somatotrophic and lactotrophic cells from neonatal animals should be additionally studied. Nevertheless, from our results we may conclude that cGMP is unlikely to play any significant role in TRH-induced GH secretion or its blocking by octanol.

Although in primary confluent cultures initially isolated adenopituitary cells probably react in a nonrandom manner, reconstructing the initial *in situ* cytoarchitectonics and paracrine interaction of particular types of cells, the intercellular communication in a monolayer may differ significantly from that in cultured spherical microaggregates and *in vivo*. Therefore, in future experiments the role of gap junction-mediated intercellular communication in the regulation of the secretory activity of pituitary cells should be investigated using octanol and nontraditional (nonmonolayer) pituitary cell cultures. According to our previous results obtained

on hepatocyte cultures [8], cultivation on a collagen substrate or within a collagen gel has a marked modifying effect on cell properties. As follows from our earlier observations [3], adenohypophyseal cells, after being seeded on a collagen substrate, reaggregate in larger 3-dimensional clusters. It seems likely that the number of pituitary cells connected through gap junctions could be changed by varying the extent of aggregation.

It could be interesting to carry out further investigations with octanol on pituitary cultures obtained from animals with long-term hormonal premedication *in vivo*, for instance, from rats with estrogen-induced pituitary hyperplasia. Such experiments could provide some clues to our understanding of the age-related pathogenetic mechanisms underlying hyperplasia and hypophyseal adenomas [11]. Such an approach is supported by data on weaked gap junction-mediated intercellular communication in carcinogenesis [16].

Summarizing the obtained results, it should be emphasized that the octanol- (or heptanol-) based express-testing system proposed by us and other researchers [4,12] may open up new perspectives for studying the role of gap junctions in the regulation of the functional and proliferative activity of cultured cells.

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