of the duration of their mitotic cycle. In this connection the fact is worth mentioning that during intestinal regeneration the increase in mitotic activity of the epitheliocytes is never as high as during regeneration of organs normally characterized by low proliferative activity [5].

The increase in the number of lymphocytes in the intestinal mucosa of the experimental recipients is evidently involved in realization of the property of lymphoid cells of stimulating proliferation, but their genesis and mechanism of action are still unknown.

LITERATURE CITED

1. A. G. Babaeva, N. A. Kraskina, and L. D. Liozner, Tsitologiya, No. 12, 1511 (1969).
2. A. G. Babaeva, N. A. Kraskina, and L. D. Liozner, Byull. Éksp. Biol. Med., No. 2, 78 (1973).
3. I. I. Dolgushin, Patol. Fiziol., No. 6, 30 (1978).
4. I. F. Kolpashchikova, Byull. Éksp. Biol. Med., No. 4, 410 (1978).
5. L. D. Liozner (ed.), Advances in the Study of Regeneration [in Russian], Moscow (1977).
6. M. E. Pliskin and R. T. Prehn, J. Reticuloend. Soc., 17, 290 (1975).
7. M. Fox and H. E. Wahman, Invest. Urol., 5, 521 (1968).

EFFECT OF CYCLIC NUCLEOTIDES ON SENSITIVITY OF EARLY SEA URCHIN EMBRYOS TO CYTOTOXIC NEUROPHARMACOLOGICAL DRUGS

Yu. B. Shmukler, G. A. Buznikov, N. G. Grigor'ev, and L. A. Mal'chenko

UDC 591.315:576.355.1

KEY WORDS: embryo; cyclic nucleotides; sensitivity.

In many cases the action of neurotransmitters has been shown to involve participation of corresponding cyclic nucleotides [11]. Substances identical with or related to neurotransmitters [1, 3, 4] and all components of cyclase systems [13] also are present in the cells of early embryos of different groups of animals (sea urchins have been best studied in this respect). It has been suggested [3, 9, 12] that these systems in early embryos, just as in differentiated cells, are functionally linked.

This hypothesis, verification of which was the aim of this investigation, was supported by the results of the writers' previous experiments, which showed that exogeneous cAMP and cGMP can reduce the sensitivity of sea urchin embryos to the specific cytotoxic action of neuropharmacologic drugs, namely antagonists of "prenerve" transmitters [6], to some degree. It has also been shown that the action of these drugs on times of micromere formation in embryos of the sea urchin *Schaphechinus mirabilis* is antagonistic relative to the corresponding effects of dibutyryl-cAMP and the phosphodiesterase inhibitor papaverine [9, 10].

EXPERIMENTAL METHOD

The test objects were early embryos of sea urchins *Strongylocentrotus intermedius* and *Scaphechinus mirabilis* (Sea of Japan), and *Arbacia lixula* and *Paracentrotus lividus* (Adriatic Sea). The technique of obtaining the gametes and incubation of the embryos was standard [5].

Antiserotonin drugs indocarb and 5-bromotryptamine [7], the tricyclic antidepressant melipramine, dibutyryl-cAMP, dibutyryl-cGMP, cAMP, the adenylate cyclase activator sodium fluoride, papaverine, and serotonin were used. The drugs were added to the incubation medium 3-5 min after fertilization. Their effects were assessed by their ability to block the first cleavage division or to abolish such a block caused by neuropharmacologic drugs, and also,

N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 354-355, March, 1984. Original article submitted March 6, 1983.

0007-4888/84/9703-0348$08.50

TABLE 1. Effect of cAMP and Dibutyryl Analogs of cAMP and cGMP on Sensitivity of Early Embryos of Sea Urchin *S. intermedius* to Neuropharmacologic Drugs (M ± m)

Neuropharmacologic drug	Concentration, M	Cyclic nucleotide	Concentration, mM	Protective action of cyclic nucleotides, %	Significance
Indocarb	$2,5 \cdot 10^{-5}$	cAMP	0,3	0*	—
		Dibutyryl-cAMP	0,2	+25,0±6,1**	>0,999
		Dibutyryl-cGMP	0,18	+***	>0,95
Melipramine	$1,2 \cdot 10^{-4}$	Dibutyryl-cAMP	0,2	+	>0,95
	$6,0 \cdot 10^{-5}$	cAMP	0,3	+30,0±11,2	>0,95
		Dibutyryl-cAMP	0,2	+38,0±8,3	>0,999
		Dibutyryl-cGMP	0,18	+35,0±13,5	>0,95
5-Bromotryptamine	$1,2 \cdot 10^{-4}$	cAMP	0,3	+60,0±9,1	>0,999
		Dibutyryl-cAMP	0,2	0	—
		Dibutylryl-cGMP	0,18	0	—

Legend. *) No significant effect of protector; **) increase in number of embryos passing through first cleavage division after addition of protector substance compared with control, in which no protector was added; ***) presence of protective effect established qualitatively by method of alternative analysis.

TABLE 2. Effect of NaF on Sensitivity of Early Embryos of Sea Urchin *S. intermedius* to Neuropharmacologic Drugs

Neuropharmacologic drug, M	Concentration of sodium fluoride, mM	Protective action of sodium fluoride, %	Significance
Indocarb $2,5 \cdot 10^{-5}$	10	+33±10,6**	>0,99
	5	0*	—
Melipramine			
$1,2 \cdot 10^{-1}$	10	0	—
$6,0 \cdot 10^{-5}$	10	+34±15,0%	>0,95
	5	+***	>0,95

Legend. Significance of asterisks the same as in Table 1.

in the case of indocarb, by its ability to affect total protein synthesis of the embryos [2]. The results were subjected to statistical analysis by methods of alternative analysis and significance of difference between means [8].

EXPERIMENTAL RESULTS

The results of most experiments on early *S. intermedius* embryos are summarized in Tables 1 and 2. The dibutyryl analogs of the cyclic nucleotides (Table 1) and sodium fluoride (Table 2) in many cases clearly had a significant protective action against the embryotoxic neuropharmacologic agents indocarb and melipramine, but as a rule did not affect sensitivity to 5-bromotryptamine. Conversely, cAMP had a protective action against 5-bromotryptamine, but no significant effect on sensitivity to indocarb.

Meanwhile papaverine in a concentration of $5 \cdot 10^{-5}$ M had no protective action against indocarb, i.e., blocking phosphodiesterase was evidently not enough to abolish the embryotoxic effect of disturbance of the function of "prenerve" mediators. Similar results were obtained in preliminary experiments on the other three species of sea urchins.

In cases where sensitivity to indocarb was determined by its ability to inhibit total protein synthesis in the embryos, no significant protective action of dibutyryl analogs of cyclic nucleotides could be demonstrated. These findings support the earlier hypothesis [1, 4] that the mechanisms of action of "prenerve" transmitters or of their analogs and antagonists on cell division and on protein synthesis are not identical.

The main result of this investigation was thus to establish that sensitivity of early sea urchin embryos to cytotoxic neuropharmacologic drugs is depressed by cyclic nucleotides and by sodium fluoride. The protective action of these substances was expressed differently in experiments with different drugs, possibly due to differences in mechanisms of transport,

binding, and intracellular reception of these preparations. Since the neuropharmacologic drugs used in this investigation are known to specifically disturb regulatory functions of "prenerve" transmitters (monoamines), participation of endogenous cyclic nucleotides in realization of the functions of biogenic monoamines can be postulated in early sea urchin embryos. The unique character of this participation, compared with that established for cell differentiation in multicellular animals is that both biogenic monoamines and cyclic nucleotides exert their regulatory functions within the same cells.

LITERATURE CITED

1. G. A. Buznikov, Low-Molecular-Weight Regulators of Embryonic Development [in Russian], Moscow (1967).
2. G. A. Buznikov, Ontogenez, No. 2, 5 (1971).
3. G. A. Buznikov, in: Neurotransmitters. Comparative Aspects, Budapest (1980), p. 7.
4. G. A.Buznikov, in: Problems of Developmental Biology, G. Khrushchov, ed., Moscow (1981), p. 76.
5. G. A. Buznikov and V. I. Podmarev, in: Objects in Developmental Biology [in Russian], Moscow (1975), p. 188.
6. G. A. Buznikov and Yu. B. Shmukler, Neurochem. Res., 6, 55 (1981).
7. G. A. Buznikov, B. N. Manukhin, L. Rakic, et al., Zh. Évol. Biokhim. Fiziol., 12, 31 (1976).
8. G. F. Lakin, Biometrics [in Russian], Moscow (1973).
9. Yu. B. Shmukler, Ontogenez, 12, 404 (1981).
10. Yu. B. Shmukler and N. E. Sadokova, in: Abstracts of Proceedings of the 6th All-Union Conference of Embryologists [in Russian], Moscow (1981), p. 204.
11. P. Greengard, Cyclic Nucleotides, Phosphorylated Proteins and Nervous System, New York (1978).
12. D. McMahon, Science, 185, 1012 (1974).
13. L. I. Rebhun, Int. Rev. Cytol., 49, 1 (1977).

CHANGES IN SUBMAXILLARY GLANDS AFTER ISOPROTERENOL INJECTION INTO INTACT AND DEPANCREATIZED MICE

E. A. Shubnikova, N. N. Kuznetsova, and L. S. Pogodina

UDC 616.316.1;591.147.7

KEY WORDS: submaxillary gland; isoproterenol; diabetes; depancreatization; insulin-like protein; immunoreactive insulin.

After injections of the β-adrenomimetic isoproterenol (IP) into rats and mice an increase in weight of the submaxillary glands (SG), hypertrophy of the acinar cells and, in many cases, a decrease in size of the cells in granular zones of the salivary tubules are observed [1, 11, 15]. The character of changes in SG cells depends on the doses of IP, times after injection, age of the animals, and other factors [1]. In healthy animals injections of IP have also been shown to stimulate secretion of B cells of the pancreatic islets [12]. After intravenous injection of IP into diabetic patients the plasma immunoreactive insulin level rises [10, 14]. The question arises whether the formation of insulin-like proteins (ILP) of nonpancreatic nature is stimulated by injection of IP. It has been shown that SG of animals and man contains ILP [4-9]. In organ culture of mouse SG, synthesis of ILP in these organs has been found [2, 3]. The writers have postulated that injections of IP, inducing hyperfunction of SG, can also intensity production of ILP in them, and in diabetes, this can lead not only to in-

Department of Cytology and Histology, M. V. Lomonosov Moscow University.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 97, No. 3, pp. 355-358, March, 1984. Original article submitted April 12, 1983.

0007-4888/84/9703-0350$08.50