ROLE OF ADRENERGIC RECEPTORS IN THE MECHANISM OF THE INFLUENCE OF CATECHOLAMINES AND DOPA ON PROLIFERATION

O. I. Sukmanskii, V. I. Arkhipenko, and L. P. Cherkashina

UDC 612.6.014.2.014.46:615.357.452.015.2.615.217.24

KEY WORDS: catecholamines; dopa; adrenergic receptors.

Data in the literature on the effect of catecholamines on proliferative processes are contradictory [8, 9] and ambiguous. Besides the inhibitory action of adrenalin and isoproterenol on cell multiplication [1, 6, 8], their stimulating effect on proliferative processes in some epithelial tissues also has been demonstrated [9]. The stimulating effect of L-3,4-dioxyphenylalanine (dopa) on repair processes in neurogenic dystrophy has been established experimentally [2]. However, the question of through which adrenoreceptors catecholamines exert their action on cell proliferation has received little study [10, 11].

Adrenalin, isoproterenol, and also dopa, under similar conditions, are known to inhibit cell proliferation in culture [3, 7, 12]. Accordingly, in the investigation described below the effect of catecholamines and dopa on cell proliferation in culture was studied with the use of adrenoreceptor blockers.

EXPERIMENTAL METHOD

Experiments were carried out on a monolayer transplantable culture of mouse fibroblasts of the L line. Catecholamines and dopa in a dose of 10^{-6} M were added to the nutrient medium 24 h after seeding of the culture. The α-adrenoblocker phenoxybenzamine and the β-adrenoblocker propranolol were added to the nutrient medium 10-20 min before the adrenergic substances, since they bind rapidly with adrenoreceptors [4], in a dose of 10^{-5} M. The adrenoblockers themselves, in the dose chosen, did not affect cell proliferation in these experiments. Proliferative processes were studied by calculation the mitotic index (in ‰), and also autoradiographically with the aid of labeled precursors of DNA and protein synthesis, namely [^{3}H]thymidine (3.7×10^4 Bq/ml) and [^{3}H]leucine (7.4×10^4 Bq/ml), respectively. Autoradiographs for microscopic study were prepared by the standard method [5]. For quantitative analysis of the autoradiographs the number of grains of silver above labeled cells or nuclei was counted. The experimental results were subjected to statistical analysis by the Fisher–Student test.

EXPERIMENTAL RESULTS

The results showed (Fig. 1) that preliminary treatment of the culture with the α-adrenoblocker phenoxybenzamine did not change the inhibitory action of catecholamines on cell proliferation. Meanwhile addition of the β-adrenoblocker propranolol prevented the inhibition of mitotic activity by isoproterenol and, to a rather lesser degree, by adrenalin. The inhibitory action of adrenalin was completely prevented by combined addition of propranolol and phenoxybenzamine. The decrease in the mitotic index of the culture as a result of the action of dopa was independent of preliminary addition of the adrenoblockers, and accordingly dopa was not used in the subsequent experiments.

The writers showed previously that the inhibitory action of catecholamines on cell proliferation in culture is connected with their influence on macromolecular synthesis, on which proliferation is based [3]. Accordingly, in the present investigation the intensity of incorporation of labeled precursors into DNA and protein of the cells under the influence of catecholamines was studied after preliminary addition of adrenoblockers to the culture.

Department of Pathological Physiology and Department of Histology and Embryology, Dnepropetrovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 7, pp. 89-91, July, 1984. Original article submitted July 21, 1983.

0007-4888/84/9807-0961$08.50

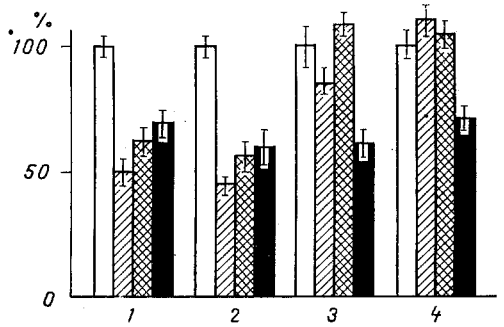

Fig. 1. Effect of adrenalin, isoproterenol, and dopa on mitotic index of culture of L cells 6 h after beginning of exposure against the background of adrenoreceptor blockade (in % of control). Unshaded columns — control; obliquely shaded columns — adrenalin, cross-hatched — isoproterenol, black columns — dopa. 1) Without adrenoblockers; 2) combined addition with phenoxybenzamine; 3) combined addition with propranolol; 4) combined addition with phenoxybenzamine and propranolol.

TABLE 1. Effect of Catecholamines on Intensity of Incorporation of Labeled Precursors into Cell DNA and Proteins after Combined Addition with Phenoxybenzamine and Propranolol (M ± m)

Experimental conditions	Mean number of grains of silver above nucleus after pulse labeling with [^{3}H]thymidine	Mean number of grains of silver above cell after pulse labeling with [^{3}H]leucine
Control	43,6±2,48	67,2±3,64
P_2	<0,01	<0,01
P_3	<0,01	<0,01
Phenoxybenzamine	40,4±2,36	62,6±3,47
P_1	<0,5	<0,5
Propranolol	39,2±2,14	63,8±3,26
P_1	<0,5	<0,5
Adrenalin	22,8±1,83	38,4±2,57
P_1	0,01	<0,01
Adrenalin + phenoxybenzamine	20,6±1,37	48,8±2,39
P_1	<0,01	<0,05
P_2	<0,5	<0,05
Adrenalin + propranolol	36,5±1,54	54,6±3,18
P_1	<0,1	<0,1
P_2	<0,01	<0,01
Adrenalin + phenoxybenzamine + propranolol	40,2±2,72	64,5±3,49
P_1	<0,5	<0,5
P_2	<0,01	<0,01
Isoproterenol	28,3±1,74	42,3±2,86
P_1	<0,01	<0,01
Isoproterenol + phenoxybenzamine	25,2±1,84	39,6±2,38
P_1	<0,01	<0,01
P_3	<0,5	<0,5
Isoproterenol + propranolol	42,7±2,36	58,6±3,42
P_1	<0,5	<0,2
P_3	<0,01	<0,01
Isoproterenol + phenoxybenzamine + propranolol	42,0±2,43	64,7±3,72
P_1	<0,5	<0,5
P_3	<0,01	<0,01

Legend. P_1) Significance of differences compared with control; P_2) compared with adrenalin; P_3) compared with isoproterenol.

The results of investigation of macromolecular synthesis under the influence of catecholamines in experiments with addition of adrenoblockers showed (Table 1) that preliminary addition of phenoxybenzamine did not prevent the decrease in incorporation of labeled precursors into cell DNA and proteins under the influence of catecholamines, as shown by counting the number of grains of silver above the nuclei and cells, respectively. Addition of propranolol prevented the fall in the level of incorporation of labeled precursors into the cells under the influence of isoproterenol and also prevented manifestation of the inhibitory action of adrenalin, but by a rather lesser degree. The inhibitory effect of adrenalin on incorporation of labeled precursors of DNA and protein was more completely prevented by the combined addition of phenoxybenzamine and propranolol.

The results thus indicate the leading role of β-adrenoreceptors in the mechanism of action of catecholamines on proliferative processes. Meanwhile the action of adrenalin, which unlike the β-adrenomimetic isoproterenol, is a stimulator of both α- and β-adrenoreceptors, can evidently be exerted not only through β-adrenoreceptors. Considering data in the literature showing that binding of catecholamines with β-adrenoreceptors is accompanied by activation of the adenylate cyclase system and subsequent cAMP formation [4], it can be tentatively suggested that the effect of catecholamines on proliferation is mediated through the cAMP system. However, the results do not rule out other possible pathways of the action of catecholamines on intracellular processes. This is a problem for further study.

Dopa, the precursor for catecholamine synthesis, like catecholamines themselves, has an inhibitory action on cell proliferation, but it is evidently not connected with adrenoreceptor activation. This hypothesis is confirmed by data in the literature indicating that dopa does not interact with binding sites of plasma membranes of turkey erythrocytes, with which either β-agonists or β-antagonists can interact [4]. Our own data for the effect of dopa on cell proliferation show that the precursor of catecholamine synthesis can exert an independent action on tissue metabolism, and not only through catecholamine formation; this is a problem which requires further study in connection with the use of dopa for the correction of trophic disorders [2].

LITERATURE CITED

1. I. A. Alov, Usp. Sovrem. Biol., 86, No. 2, 216 (1978).
2. S. V. Anichkov, The Selective Action of Mediators [in Russian], Leningrad (1974).
3. V. I. Arkhipenko, O. I. Sukmanskii, and L. P. Cherkashina, in: Abstracts of Proceedings of the 11th Congress of the Ukrainian Physiological Society [in Russian], Kiev (1982), p. 17.
4. R. Lefkovits, in: Interaction between Hormones and Receptors [in Russian], Moscow (1979), p. 324.
5. O. I. Epifanova, V. V. Terskikh, and A. F. Zakharov, Autoradiography [in Russian], Moscow (1977).
6. Yu. A. Romanov and A. N. Blokhina, Byull. Éksp. Biol. Med., No. 9, 94 (1971).
7. O. I. Sukmanskii and L. P. Cherkashina, in: Current Problems in Pathology [in Russian], Baku (1982), p. 409.
8. T. L. Boshell and C. Pennington, Cell Tissue Res., 213, 411 (1980).
9. E. R. Burns, Growth, 42, 333 (1978).
10. H. Hunt and P. J. M. Tutton, Clin. Exp. Pharmacol. Physiol., 3, 207 (1976).
11. W. R. McInnes and P. J. M. Tutton, Arch. Pathol. Anat. Abt. B., 21, 341 (1976).
12. O. I. Sukmanskii, R. D. Barabash, P. S. Varecha, et al., in: Abstracts of the 3rd International Congress of Pathological Physiology, Varna, Bulgaria (1978), p. 224.

TESTICULAR INHIBIN-LIKE FACTOR OF FETAL AND NEWBORN RATS

O. I. Brindak, B. V. Aleshin, and A. B. Malyshev

UDC 612.616

KEY WORDS: testes; inhibin-like factor.

The problem of the existence of a factor limiting growth of an organ or maintaining the state of equilibrium in constantly renewed populations has engaged the attention of embryologists, endocrinologists, and clinicians for a long time. We know that during the prenatal period of mammalian ontogeny active processes of proliferation of the genocytes take place with preparation for meiosis. However, in some species at the end of pregnancy, and in others during the first days after parturition, proliferation of the primary sex cells is inhibited, as also is their entry into meiosis, until the period of puberty. The discussion on which factors affect this process still continues and opinions of investigators are extremely contradictory. An inhibin-like substance, concerned in the regulation of spermatogenesis and also in maturation of follicles in adult humans and mammals has been isolated [2, 5, 6, 8, 9, 11]. The authors cited state that there is more inhibin in the testes and ovaries of sterile men and women than in subjects with normal fertility. However, the question of the existence of an inhibin-like factor in prenatal and early postnatal periods of human and mammalian ontogeny, which could exert its action on the gonocytes, remains debatable.

Laboratory of Pathomorphology, Khar'kov Research Institute of Endocrinology and Hormone Chemistry. Research Institute of Biology, A. M. Gor'kii Khar'kov University.

(Presented by Academician of the Academy of Medical Sciences of the USSR L. T. Malaya.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 7, pp. 91-93, July, 1984. Original article submitted July 22, 1983.

0007-4888/84/9807-0963$08.50