REGULATORY ROLE OF DOPA AND COMPONENTS OF THE CYCLIC AMP SYSTEM

I. I. Ivanov,* I. S. Gavrilenko,
R. A. Zarembskii, V. N. Bobrova,
L. N. Rumyantseva, and T. M. Bulyko

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Injection of nonradioactive dopa (1 mg/mouse) before injection of [^{3}H]dopa (20 μCi/mouse) into albino mice with Harding–Passy melanoma leads to increased accumulation of tritium in the tumor tissue. Radioactivity of the melanoma in this experimental group was twice as high as the radioactivity of the tumor tissue in animals receiving injections of [^{3}H]dopa alone. Investigation of adenylate cyclase and phosphodiesterase activity and the cyclic AMP level in the melanoma of the mice 2 h after injection of dopa (1 mg/mouse) revealed accumulation of cyclic AMP and increased phosphodiesterase activity; adenylate cyclase activity was depressed. It is suggested that dopa exerts its effect not only as a precursor of melanin, but also through the cyclic AMP system, affecting the activity of enzymes of melanogenesis.

KEY WORDS: Harding–Passy melanoma; dopa; cyclic AMP; stimulation of melanogenesis; phosphodiesterase; adenylate cyclase.

Previous investigations confirmed that 3,4-dihydroxyphenylalanine (dopa) can be used as precursor of melanin synthesis for the creation of new radioactive chemothermapeutic agents [2, 5]. Dopa has been shown to transmit a radioactive label – ^{14}C or ^{3}H – into the Harding–Passy melanoma [3, 4, 6]. Dopa is thus an important substrate for melanin synthesis. On the other hand, dopa can evidently behave as a regulator in the biochemical processes of melanogenesis. It has been suggested that it acts as an allosteric effector of the activity of tyrosinase – a key enzyme of melanogenesis [11]. The mechanism of this allosteric effect has not been studied. It is possible that it may be realized by means of intermediate metabolites.

The exceptional role of the cyclic AMP system as an intermediary in the action of many hormones and biologically active substances on cell metabolism has recently been demonstrated in several publications. In particular, the participation of the cyclic AMP system in melanogenesis has been reported. A direct connection has been shown, for instance, between a high level of cyclic AMP and increased tyrosinase activity [9-14]. These experiments, it must be emphasized, were carried out in vitro only. In its chemical structure dopa is close to adrenalin, which exerts its effect through the participation of the cyclic AMP system. Attention is therefore drawn to the importance of an experimental study of the effect of preliminary administration of dopa on melanogenesis in vivo and also of the effect of dopa on the components of the cyclic AMP system in the melanoma.

EXPERIMENTAL METHOD

A pigmented melanoma of the Harding–Passy strain was transplanted subcutaneously into noninbred albino mice. The animals were used in the experiments 4 weeks after transplantation of the tumor, when the nodules measured on average 0.5 × 0.5 × 0.5 cm. There were two series of experiments: in series I the effect of preliminary injection of nonradioactive dopa on the incorporation of radioactive dopa in the melanoma was studied; in series II the effect of injection of nonradioactive dopa on the state of the cyclic AMP system in the tumor was examined.

In the experiments of series I the animals were divided into two groups: the 10 mice of the control group received [^{3}H]dopa by intraperitoneal injection in a dose of 20 mCi/mouse, and the 10 mice of the experimental

*Deceased.

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TABLE 1. Effect of Injection of Nonradioactive Dopa on Tritium Accumulation in Harding–Passy Melanoma after Injection of [^{3}H]dopa and on Components of Cyclic AMP System (in all cases [^{3}H]dopa injected 1 h before the investigation)

Series of experiments	Numbers of animals	Index studied	Control (no nonradioactive dopa given)	Experiment (2 h after injection of nonradioactive dopa)	P
I	10	Radioactivity, μCi/mg dry weight of protein	$38 \cdot 10^{-3}$	$84 \cdot 10^{-3}$	—
II	10	Cyclic AMP content, pmoles/g	171,0±14,9	247,9±20,8	<0,05
		Phosphodiesterase activity, nmoles cyclic AMP/min/mg protein	0,66±0,20	1,35±0,25	<0,001
		Adenylate cyclase activity, pmoles cyclic AMP/min/mg protein	20,8±1,77	12,7±1,4	<0,002

group received an injection of nonradioactive dopa in a dose of 1 mg/mouse followed 1 h later by an intraperitoneal injection of 20 mCi [^{3}H]dopa per mouse. The animals of both groups were decapitated 1 h after the injection of [^{3}H]dopa. Peripheral regions of the tumor, equal in weight, were pooled in each group separately and prepared for ignition by Schoninger's method [13]. Radioactivity was determined in the ash by means of a liquid scintillation counter [7]. These measurements of radioactivity were mean values obtained from the group of animals at each time.

In the experiments of series II the animals with tumors also were divided into two groups, control and experimental, and the animals of the experimental group had received dopa in a dose of 1 mg per mouse 2 h before investigation. Pieces of melanoma were quickly removed from the animals of the control and experimental groups, immersed in liquid nitrogen and, after appropriate treatment of the samples, activity of adenylate cyclase and phosphodiesterase and the cyclic AMP content in them were determined – adenylate cyclase activity by the method of Drummond and Duncan [8], phosphodiesterase activity by means of the Ferment-1 apparatus by a conductometric method [1]. The cyclic AMP level was determined by means of the kit of ready-made reagents from the Radiochemical Centre, Amersham (England).

EXPERIMENTAL RESULTS

The results of the control group of series I show that the Harding–Passy melanoma actively accumulates tritium after injection of [^{3}H]dopa: 1 h after injection of the compound the radioactivity of the tumor was $38 \cdot 10^{-3}$ μCi/mg. In the experimental group, in which nonradioactive dopa was injected before the radioactive form, the radioactivity of the tumor was much higher, namely $84 \cdot 10^{-3}$ μCi/mg.

The results of the experiments of series II showed that preliminary injection of dopa into animals with tumors increased the cyclic AMP accumulation in the melanoma (247.9±20.8 pmoles/g compared with 171.0±14.9 pmoles/g in the control; Table 1). A statistically significant ($P < 0.001$) increase in phosphodiesterase activity (from 0.66±0.20 nmoles cyclic AMP/min/mg protein in the control to 1.35±0.25 nmole cyclic AMP/min/mg protein in the experiments). Meanwhile the adenylate cyclase activity in the melanoma was depressed (from 20.8±1.77 pmoles cyclic AMP/min/mg protein in the control to 12.7±1.4 pmoles cyclic AMP/min/mg protein in the experiment).

These results confirm the existing view that dopa is a precursor utilized in melanin biosynthesis, and it thus participates in melanogenesis. Preliminary injection of nonradioactive dopa stimulates the incorporation of radioactivity into the tumor from the same dose of radioactive dopa ([^{3}H]dopa), i.e., it exerts a "regulatory" rather than a "substrate" effect. It also follows from these results that the components of the cyclic AMP system play an important role in melanogenesis. The cyclic AMP level is raised 2 h after injection of dopa, so that phosphodiesterase activity is evidently induced [10]. Depression of adenylate cyclase activity observed during this period and the at first glance contradictory increase in the cyclic AMP content can be explained by the action of cyclic AMP as a modulator of adenylate cyclase repressor [12].

LITERATURE CITED

1. V. S. Andreev, L. Yu. Ketrits, B. F. Korovkin, et al., Lab. Delo, No. 2, 95 (1976).
2. V. M. Vdovenko et al., Dokl. Akad. Nauk SSSR, 164, 95 (1965).
3. V. M. Vdovenko et al., Radiobiol. Radioter. (Berlin), No. 1, 49 (1968).
4. V. M. Vdovenko et al., Vopr. Onkol., No. 7, 67 (1970).
5. I. I. Ivanov, A. L. Solov'ev, and I. S. Gavrilenko, Vopr. Onkol., No. 6, 82 (1964).
6. I. I. Ivanov, V. M. Vdovenko, V. N. Bobrova, et al., Vopr. Onkol., No. 8, 50 (1972).

7. I. I. Ivanov, V. K. Modestov, Yu. M. Shtukkenberg, et al., Radioactive Isotopes in Medicine and Biology [in Russian], Moscow (1955).
8. I. I. Drummond and L. Duncan, J. Biol. Chem., 245, 976 (1970).
9. T. Lee, M. Lee, and M. L. Lu, Endocrinology, 91, 1180 (1972).
10. I. Pastan and R. Perlman, Science, 169, 339 (1970).
11. S. H. Pomerantz and M. C. Warner, Biochem. Biophys. Res. Commun., 24, 25 (1966).
12. E. W. Sutherland, R. Ho, T. Russell, et al., Metabolism, 24, 257 (1975).
13. G. Wong, Y. Pawelek, M. Sansone, et al., Nature, 248, 351 (1974).

EFFECT OF TRIORTHOCRESYL PHOSPHATE POISONING ON INTENSITY OF INCORPORATION OF [2-^{14}C]ACETATE INTO PHOSPHOLIPIDS AND CHOLESTEROL OF THE GUINEA PIG BRAIN AND SPINAL CORD

N. P. Taranova

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A severe form of chronic triorthocresyl phosphate poisoning was induced in guinea pigs by a single intradermal injection of this compound and the intensity of incorporation of [2-^{14}C]acetate into lipids of the spinal cord and brain stem was investigated in vivo. In the paralytic stage of the disease incorporation of the label into phospholipids and cholesterol was clearly reduced; inhibition of synthesis of these lipids was observed not only in the most vulnerable lumbosacral region of the spinal cord, but also in the brain stem, evidence of the systemic character of the disturbance of lipid metabolism in the CNS and of changes in the metabolism of the oligodendroglia.

KEY WORDS: phospholipids; cholesterol; brain and spinal cord; triorthocresyl phosphate poisoning.

Chronic poisoning in man by certain organophosphorus compounds (OPC) widely used in agriculture and industry [1, 3, 4], leading to severe damage to the nervous system with the development of permanent pareses and paralyses of the limbs, has been described in the literature [9, 11]. The neurotoxic effects of these compounds are unconnected with their anticholinesterase properties [10]. Clinical and morphological studies have shown that the neuroparalytic action of these OPC is connected with their demyelinizing action [2, 10], but the mechanism of this phenomenon still remains virtually unstudied.

Existing fragmentary investigations of the lipid components of myelin are contradictory and do not give a complete picture of the action of the demyelinizing OPC on the lipid composition of nerve tissue.

The object of this investigation was to study disturbances of phospholipid and cholesterol metabolism of nerve tissue in vivo by the use of [2-^{14}C]acetate as radioactive precursor of their synthesis during chronic triorthocresyl phosphate (TOCP) poisoning.

EXPERIMENTAL METHOD

An experimental model of chronic poisoning was created in adult male guinea pigs weighing 350-450 g by intradermal injection of TOCP (the industrial oily mixture, containing 37% of the ortho isomer) in a dose of 2.0-2.2 ml/kg body weight. [2-^{14}C]acetate in a dose of 100 μCi/100 g body weight was injected subcutaneously 27-33 days after injection of TOCP. The animals were decapitated 2 h later, and the brain stem and lumbosacral region of the spinal cord were removed.

Extraction of lipids from the tissue homogenate, isolation of cholesterol and the total phospholipid fraction from the total lipid extract, and also their quantitative determination was carried out by the method de-

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