CONTENT OF PROSTAGLANDINS AND CYCLIC AMP IN RAT TISSUE

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The content of prostaglandins (PG) and cyclic adenosine-3',5'-monophosphate (cyclic AMP) in rat tissues was studied by a radioisotope method. The maximal content of both PG and cyclic AMP was found in the same organs, those functioning most actively: the brain, endocrine glands, and small intestine, and the minimal content in adipose tissue. The investigations show close functional interdependence between PG synthesis and adenylate cyclase activity in the tissues of the body.

KEY WORDS: *prostaglandins; cyclic AMP, radioimmunological determination in the tissues.*

The role of prostaglandins (PG) in various physiological processes *in vivo* can be explained by their stimulating effect on adenylate cyclase, leading to the formation of cyclic adenosine-3',5'-monophosphate (cyclic AMP) [1, 10]. However, there are data in the literature which conflict with the view that there is a single mechanism of action of PG [3]. For instance, PG act on the thyroid and adrenal glands and the ovaries like tropic hormones and cause an increase in the cyclic AMP content [5, 11, 13]. In adipose tissue, on the other hand, PG inhibit the formation of cyclic AMP stimulated by hormones [6]. Nevertheless there is no doubt about the interconnection between PG-E and cyclic AMP and their joint role in the mechanism of hormonal action [7].

Sensitive enzymic [9] and radioisotope methods have recently been developed for determining the levels of PG and cyclic AMP in the tissues and blood. There have been very few comparative investigations of the content of PG and cyclic AMP in vitally important organs. Although investigations of the concentration of PG and cyclic AMP [2] in the blood give some idea of the role of these biologically active substances in pathogenesis of certain diseases, they cannot be used to study the character of the interaction between these substances in the tissues.

The object of this investigation was to determine the content of PG-B (equivalent to the sum of PG-A_1 and PG-E_1, and also a certain quantity — about 20% — of PG-A_2 and PG-E_2) and of cyclic AMP in rat tissues.

EXPERIMENTAL METHOD

In experiments on sexually mature male rats aged 8-10 months and weighing 210-360 g the tissues of the cerebral hemispheres, cerebellum, heart, skeletal muscles, liver, kidneys, lungs, small intestine, spleen, testes, epididymal fatty areolar tissue, and the thyroid and adrenal glands and blood were used.

The content of PG-B in the tissues was determined by a radioimmunological method using kits obtained from the firm "Clinical Assay Inc." (USA). The method is based on the principal of competitive binding of specific antiserum against PG-B_1 with the PG con-

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TABLE 1. Content of PG-B and Cyclic AMP in Rat Tissues (M ± m)

Organs and tissues	PG-B, per gram tissue	Cyclic AMP, pmoles/g tissue
Brain (hemispheres)	138,4±16,4	1246±239
Cerebellum	91,8±42,5	—
Heart	16,1±5,0	631±27
Skeletal muscle	10,9±1,8	405±32
Liver	22,3±1,6	435±58
Kidneys	23,8±6,9	449±27
Lungs	53,8±6,8	—
Small intestine	92,3±5,7	935±100
Spleen	5,0±1,2	—
Testes	20,0±4,3	—
Epididymal fat	0,93±0,23	73±12
Thyroid gland	65,8±6,8	896±90
Adrenal glands	62,9±2,4	4901±181
Blood*	1,88±0,025	9,9±2,4

*Content of substances in 1 ml, respectively.

tained in the test sample, and with labeled PG-B_1-^{3}H. A reciprocal proportional relationship exists between the content of PG-B in the tissues and the binding of labeled PG with the antiserum.

The rats were decapitated at 0-4°C. The tissues were homogenized immediately after sacrifice. In the interval between sacrifice and homogenization the tissues were kept at −20°C; 100 mg of tissue was homogenized in 1 ml of Na-phosphate buffer and 3 ml of a mixture consisting of isopropanol, 0.2 N HCl, and ethyl acetate (3:1:3). The samples were then treated with 2 ml ethyl acetate and 3 ml distilled water, mixed, and centrifuged. The organic phase, containing PG, was separated and the ethyl acetate evaporated off at 55°C. To estimate the loss of PG during extraction, parallel with the test tissues PG also was extracted from preparations containing a known quantity of PG-B-^{3}H. The percentage loss, which was sufficiently stable (24-28), was taken into account when the absolute content of PG-B in the tissues was determined. The dry residue was treated with 2 ml of buffer solution. To convert PG-E and PG-A into PG-B, 0.2 ml of 1 N NaOH (pH 12,2-12.9) was added to the solution which was kept on a boiling water bath for 5 min, after which the pH was adjusted to 7.4 with glacial acetic acid. Next, 0.4 ml of the sample was transferred to a test tube containing 0.6 ml buffer, to which 0.05 ml of PG-B-^{3}H and 0.05 ml of rabbit antiserum against this PG were added. After incubation for 60 min at 37°C 0.1 ml of normal rabbit serum and of goat antirabbit serum was added to the solution to form a precipitate which bound the PG—antiserum complex. After removal of the supernatant by centrifugation the radioactivity of the residue was determined with the "Isocap-300" liquid scintillation system (Nuclear Chicago). A standard curve was plotted by the use of definite quantities of stable PG-B and it was used to determine the absolute content of PG-B in 100 mg of tissue.

The concentration of cyclic AMP also was determined by radioisotope analysis, using kits obtained from the Radiochemical Centre, Amersham (England). Cyclic AMP was extracted from the tissues by the method of Steiner et al. [12]. The extract (50 µl) was transferred to test tubes containing 0.05 ml cyclic AMP-^{3}H and 0.1 ml binding protein. After incubation (2 h, 2-4°C) 100 µl of a suspension of carbon was added to the solution which was centrifuged at 2500 g. Next, 200 µl of the sample from each tube was transferred to flasks containing scintillation fluid for radiometric investigation. A standard curve was plotted from data for the radioactivity of samples containing a known quantity of cyclic AMP in 1 g of the test tissue.

EXPERIMENTAL RESULTS

As Table 1 shows, the highest content of PG-B was found in the brain and small intestine and the lowest in the epididymal fat. A high concentration of PG also was found in the endocrine glands and lungs. There are no data in the literature for the PG-B content in the tissues of rats. The quantity of PG-E_2 determined by gas chromatography [8] in the lungs, heart, kidneys, spleen, and ovaries is a little less than that obtained in the present investigation; this is natural because PG-B is equivalent not only to PG-E, but also

PG-A. Meanwhile the level of PG found in the present experiments in the brain tissues is more than ten times higher than that found by Juvenas [8], and the difference cannot be explained in this way.

The results obtained for the content of cyclic AMP (Table 1) in the brain [4, 12], kidneys, and liver [4], adipose tissue [12], and thyroid gland [14] agree with data in the literature. The highest content of cyclic AMP in the tissues studied was found in the adrenals. The mean blood concentration of cyclic AMP in rats was a little lower than in man [12].

The maximal content of both PG and cyclic AMP was thus found in the same organs, those with the most active function, namely the brain, endocrine glands, and small intestine; the minimal content was found in adipose tissue. These investigations confirm the close functional interdependence of PG synthesis and adenylate cyclase activity in the tissues of animals.

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