Serum and Bile Modifications in the Guinea-Pig Following Chronic Treatment with PGE_1 and PGE_2

L. R. RODRIGO, F. POZO, B. MARIN and O. SCHIAFFINI[1]

Department of Physiology, Medicine and Sciences, University of Oviedo (Spain); and University College of Las Palmas, School of Medicine, Apartado de Correos 550, Las Palmas de Gran Canaria (Spain), 29 December 1975.

Summary. PGE_1 increases cholesterolemia without lipemia modifications. In bile there are not modifications in cholesterol levels and total lipids appear diminished. PGE_2 raise the lipemia and have no effect in cholesterolemia, moreover bile cholesterol and total lipids exhibit no changes. Both PGE_1 and PGE_2 decreased the bile volume.

Besides the numerous studies on the different effects of prostaglandins on organs and tissues, it must be added that strong stimuli of gastric secretion, such as histamine or food, can be inhibited by the same substances[2]. The effects of prostaglandins on the digestive system are very contradictory, depending on dosage and species used as well as method of experimentation. In human, these substances produce a notable increase in the secretion of water and electrolytes in the jejunum[3]. The prostaglandins of the E series in vitro reveal a cholagogue effect in the guinea-pig[4, 5].

In the present work we study the same parameters in chronic treatment to see whether results do superimpose or, on the contrary, change depending on the method of experimentation.

Material and method. Adult male and female guinea-pig were kept under light and temperature control and were given access to food and tap water ad libitum. Before undergoing the experiments, the animals were not fed for 24 h, although they had free access to water.

Each guinea-pig received i.p. injections of prostaglandins (0.1 µg) on alternate day for 2 weeks. The control animals were injected with the vehicle (saline solution 9‰ NaCl). Both groups were anesthetized by a 20% solution of Urethane injected i.p. in doses of 1 ml/100 g of weight. Bile was obtained with a catheter placed in the choledochus, and was collected for 15 min after the surgical procedure was finished. Blood was extracted by placing a catheter in the carotid artery. The guinea-pig's temperature was followed during the whole surgical procedure, and no changes were detected. Cholesterol was determined according to ZLATKIS and ZHAN method[6] and total lipids according to ZOLLNER and KIRSCH's method[7], SNEDECOR's variance analysis[8] and Student's-test following FISHER and YATES were used in the statistical analysis[9]. Minimal significant difference of groups under study was determined by TUCKEY's multiple comparison test[10].

Results. From the Table, it can be seen that PGE_1 increases cholesterolemia, while PGE_2 does not change the normal levels of cholesterol in blood, being 11.64 the *F*-value for PGE_1. On the contrary, PGE_2 increases the levels of lipemia with an *F*-value of 29.51, and PGE_1 do not change this parameter.

In the bile, chronic administration of PGE_1 and PGE_2 does not significantly affect cholesterol values. Concentration of lipids in the bile is lowered by PGE_1, with an *F*-value of 26.43, PGE_2 had no effect. The volume of the bile itself is significantly lowered by both prostaglandins, the value of F being 11.31.

Discussion. In these groups of animals treated chronically, the anticholeretic effect of both prostaglandins agrees with that found in the acute treatment, as we had discussed according to previous observations[11, 12].

Acute in vivo experiments carried out in our laboratory showed that PGE_1 increased the levels of cholesterolemia and lipemia, lowering the levels of cholesterol, total lipids and volume of bile in guinea-pigs. PGE_2 increased cholesterolemia without lipemia modification, and like PGE_1 lowered the levels of cholesterol, total lipids and volume of bile in guinea-pigs[5, 10].

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Experimental groups	Cholesterol (mg/100 ml)		Total Lipids (mg/100 ml)		Volume (ml)
	Serum	Bile	Serum	Bile	Bile
Controls	46.15 ± 2.91	26.05 ± 1.68	393.25 ± 20.36	382.50 ± 27.32	3.89 ± 0.13
PGE_1	62.25 ± 3.49[a]	27.50 ± 2.79	389.25 ± 19.32	193.25 ± 18.23[a]	2.89 ± 0.24[a]
PGE_2	42.85 ± 2.67[a]	29.75 ± 1.28	623.00 ± 32.17[a, b]	378.30 ± 15.74[b]	2.90 ± 0.10[a]

Mean values ± SE. 20 animals per group. [a] p 0.01 vs. control. [b] p 0.01 vs. PGE_1.

But in the case of chronic treatment, the hepatic metabolic alterations described by some authors[13] had apparently reached a stage of at least partial adaptation, since hepatic excretion of cholesterol suffers no alteration and the lipid excretion is only diminished by the action of PGE_1. As regards cholesterolemia, a difference between PGE_1 and PGE_2 is observed, the former increasing and the latter not modifying that parameter. We would tend to interprete the rise of blood cholesterol caused by PGE_1 as a direct effect on its processes of bio-synthesis, since the exogenous contribution of cholesterol would be inhibited by a lesser contribution of intestinal biliary salts due to the clear anticholeretic effect.

As regards the effect on lipids, the difference between PGE_1 and PGE_2 in chronic treatment appears again. With respect to the former, our results agree with those of other authors[14], who found the antilipolytic effect of this prostaglandin. We found no variations in the blood values of total lipids. PGE_2, on the other hand, increases the blood values of total lipids without altering their biliary excretion. According to the low dosage employed for the present study, a lipolytic effect may be observed, which had been already described in another animal species administered in vivo[14]. On dealing with prostaglandins and their effects on organs and tissues, results are contradictory and even more controversial in the field of the lipid metabolism. Endogenous mechanisms, as well as the exogenous or intestinal reabsorption contributions, are disturbed by the direct effect of the prostaglandins on the intestinal muscular system[15,16].

Large contradictory contributions of experimental data attribute the effect of prostaglandins both to direct mechanisms acting on the intramural neuronal plexus[17] and to indirect mechanisms which are either dependant on oxygen or mediated by the ATP-cAMP system[18]. Disparity and variety contributions increase the interest and excite attention to this field, justifying the need of deeper studies to reach more established conclusions.

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Fluorometric Study of Interaction between ACTH Fragments and Bovine Adrenocortical Membranes[1]

T. Muraki, E. Saito and Y. Ichikawa

Departments of Pharmacology and Internal Medicine, Keio University School of Medicine, Shinanomachi, Shinjuku, Tokyo (Japan), 30 April 1976.

Summary. Corticotropin$_{1-24}$ and [Gly^1]corticotropin$_{1-18}$ amide increased the fluorescence of 1-anilinonaphthalene-8-sulfonate which bound to the bovine adrenocortical membranes. The two ACTH fragments interacted with the protein of the membranes and increased the net positive charge of the membranes.

1-Anilinonaphthalene-8-sulfonate (ANS) is a fluoroprobe which emits strong fluorescence when it binds to biological membranes, and its fluorescence is a sensitive indicator of changes in membrane charge[2]. If ACTH changes the charge of adrenocortical membranes when it binds to them, it would be possible to detect the changes of membrane charge by investigating changes in ANS fluorescence. The present study was planned to investigate whether ACTH fragments affect the net positive charge of bovine adrenocortical membranes, using ANS as a probe.

Materials and methods. Corticotropin$_{1-24}$ was obtained from Ciba, Basel, [Gly^1]corticotropin$_{1-18}$amide, [Gly^1]-corticotropin$_{1-14}$ and [Gly^1]corticotropin$_{1-10}$ were obtained from Shionogi, Osaka. Pig ACTH was purchased from Sigma. Corticotropins were dissolved at a concentration of 1 mg/ml 0.01 *N* HCl for titration. ANS (Tokyo Chemical Industry) was used as the sodium salt. Phosphatidylcholine cholinephosphohydrolase [E.C.3.1.4.3.] (phospholipase C from *Cl. welchii*) was purchased from Sigma, pronase (grade E) from Kakenkagaku, Tokyo. The plasma membranes of the bovine adrenal cortex were prepared according to the method of Finn et al.[3], and were characterized by electronmicroscopy. Membranes were suspended in 20 m*M* *Tris*-HCl, pH 7.4 containing 1 m*M* EDTA. EDTA was omitted in case of titration with $CaCl_2$. Fluorescence measurements were carried out in a Hitachi 203 spectrofluorometer. Determination of the number of binding sites for ANS and the apparent binding constants were made by Scatchard plots constructed according to Weidekamm et al.[4]. Protein concentration was determined by the method of Lowry et al.[5]. Phospholipids

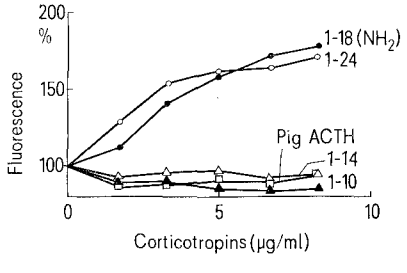

Fig. 1. Effect of corticotropin analogs on the fluorescence of ANS-adrenocortical membrane complexes. 63 µg membrane protein/ml; ANS, 76 µ*M*. The fluorescence without corticotropin analogs was set at 100%. 1–24, Corticotropin$_{1-24}$; 1–18(NH$_2$), [Gly^1]corticotropin$_{1-18}$ amide; 1–14, [Gly^1]corticotropin$_{1-14}$; 1–10, [Gly^1]corticotropin$_{1-10}$.

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