EFFECT OF EXOGENOUS CALCIUM IONS ON PROSTAGLANDINS OUTPUT FROM RAT HEPATIC TISSUE SLICES

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INTRODUCTION

The requirement of extra and/or intracellular CQ++ to stimulate prostaglandins synthesis varies in different tissues .

Poyser, (1985), elicited that prostaglandins synthesis and efflux from their active biosynthetic sites in almost tissues is dependent upon mobilization of Ca++ from intracellular stores. The extracellular Ca++ is essential for release of Ca++ from intracellular stores and essential for replenish these stores., Rilley & Poyser, (1987).

Ernest et al., (1983), reported that the activity of phospholipase seems to be increased by the presence of Ca++. PLA_2 is extremly important in the release of arachidonic acid, (which is the most abundent of prostaglandins precursors in almost all the tissues), followed by a rise of prostaglandins biosynthesis. So PLA_2 is considered as a regulating and triggering enzyme for prostaglandins biosynthesis.

Prostagladins are not stored preformed but are synthesized and released as required. Therefore the increased efflux reflects the increased biosynthesis and not release of prostglandins as endogenous constituents Ramwell & Shaw, 1970 and Olley & Coceani, (1980).

Synthesis of PGF_2 & may be derived from PGE_2 and/or from PGD_2. The interconversion between PGF_2 & and PGF_2 is dought, Yamamoto, (1983), reported that there is no a direct interconversion between PGE_2 and PGF_2 &. In contrast Ernest et al., (1983) found that reduction of the Ketogroup at C_9 in PGE_2 to form PGF_2 & compound is found to take place in guinea pig as well as in human.

The aim of the present work is to study the effect of extracellular Ca++

on PGE_2 and $PGF_2 \alpha$ output from hepatic tissue slices to elucidate the possible role of extracellular Ca++ on the prostaglandins metabolism in hepatic tissue of albino rats.

MATERIAL AND METHODS

Male albino rats weighing 150-200 gms were used in this experimental study. The rats were fed at libidum with milk, bread, carrots and all necesary vitamins and minerals.

Tissue preparation : -

The rats were scarified, the liver tissue slices were excised from the animals, and washed thoroughly with saline. The hepatic tissues were incubated in kreb's Ringer phosphate buffered solution, supplemented by 200 mg/dl glucose to maintain the life of the tissue, according to Maligieri et al., (1975).

Calcium gluconate ampoules 10% produced by Swiss pharma S. A. E. Cairo, were added to the incubation media in a dose of 29 ugm/gm wet tissue.

Assay :

After incubation in metabolic shaker for 2 hours at 37°C, the liver slices were removed and aliquots of the media were used for determination of PGE_2 and $PGF_2 \alpha$ concentraction according to Yalow and Berson (1971), which depends on the competitive binding principles of radio-immunoassay. The Kits were supplied from Dade Bater Travenol Diagnostic Inc .

Statistical analysis :

The results are expressed as $\pm$ S. E. M. The data were analysed by unpaired Student's "t" test; the null hypothesis was rejected when P value was leas than 0.05.

RESULTS

Addition of calcium gluconate solution (29 ug/gm wet tissue) resulted in a significant decrease in $PGF_2 \alpha$ with a high significant increase in PGE_2 output from the hepatic tissue slices to the incubation media.

This will be illustrated in table 1.

DISCUSSION

In this study the addition of calcium gluconate to the incubation media caused a highly significant increase in PGE_2 output from the hepatic tissue slices incubated in Kreb's Ringer phosphate buffered solution, this data is coinside with that obtained by Olsen

et al., (1983) , Poyser (1985) and Riley & Poyser (1987), they reported that prostaglandins output is stimulated by Ca++ ions. Prostaglandins synthesis and efflux from their active biosynthetic sites are dependent upon mobilization of Ca++ from intracellular stores with a direct interaction with the membranes of endoplasmic reticulum, the main site of PLA_2 and prostaglandins synthesizing enzymes. The extracellular Ca++ releases the intracellular Ca++ from its stores, and is essential for replenish these intracellular stores.

Weis & Malik (1985), elicited that activation of beta- adrenergic receptors will stimulate cardiac PGE_2 and 6-keto-$PGF_{1\alpha}$ synthesis. It is absolutly dependent on extracellular Ca++. Activation of these beta receptors increase transmembrane Ca++ influx, which by activating release of Ca++ from its intracellular stores, stimulatea release of arachidonic acid consequent to activation of phospholipase A_2, making free arachidonic acid available for PGs synthesis.

Recently it has been reported that 6-keto $PGF_{1\alpha}$ synthesis in the rat aortic rings elicited by norepinephrine was abolished completely by removal of Ca++ from the mediam and by Ca++ channel blockers, verapamil and nifedipine Stewart et al., 1984).

Cooper and Malik (1986), concluded that norepinephrine requires extracellular as well as intracellular Ca++ to express its rnaximal effect on renal PGs synthesis.

Ca++ that is released from intracellular sites interact with calmodulin. By apecific calmodulin antagonist, rendering calmodulin biologically inactive, basal and stimulated PGE_2 efflux markedly inhibited. In addition calmodulin antagonists might also inhibit PGE_2 efflux by interfering with Ca++-phospholipid sensetive protein kinase C. Activation of protein Kinase C might activate release of Ca++ from intracellular stores, thus inducing PGE_2 output, Levis & Weiss, (1976) Picket et al., (1977) and Cooper, & Malik , (1986).

Broekemeier et al., (1985), showed that verapamil exerted a moderate degree of phospholipase A_2 inhibitory activity, whereas the other calcium channel blockers, diltiazem and nifedipine, exhibited only weak inhibition of rat liver mitochondrial phospholipase A_2 activity.

Recently Danon et al., (1986), reported that verapamil, at different concentrations, exerts a dual action on

cellular phospholipase A_2 activity, thereby stimulating action at low concentration which is extracellular Ca++ dependent and inhibiting action at high concentration and consequently on PGE_2 synthesis by hydronephrotic interstitial cells. While the other calcium channel blocker nifedipine and diltiazem failed to stimulate PGE_2 synthesis.

Addition of calcium gluconate to the incubation media resulted in a highly significant decrease in $PGF_2 \alpha$ output, this decrease can be attributed to one or more of the following :

1- decrease the synthesis of $PGF_2 \alpha$ from PGD_2.

2- increase the conversion of $PGF_2 \alpha$ to PGE_2 via stimulation of 9-hydroxydehydrogenase enzyme.

3-decrease the conversion of PGE_2 to $PGF_2 \alpha$ through inhibition of PG-9-Keto-reductase enzyme. Extracellular Ca++ ions may have a role on the activity of these enzymes. This point must be studied in detail in further investigation.

SUMMARY AND CONCLUSION

This vitro study was an attempt to study the possible role of Ca++ ions on metabolism of prostaglandins in hepatic tissue of albino rats.

Addition of calcium ions in the from of calcium gluconate 10% ampoule in a dose of 29 ug/gm wet tissuse to the Kreb's Ringer phosphate media cause a high significant increasre in PGE_2 output from hepatic tissue slices. This is associated with a significant decrease in $PGF2\alpha$. These fingings may give an additional information on the possible role of Ca++ ions on metabolism of prostaglandins.

From the present study, it is concluded that addition of extracellular Ca++ ions to the incubation media resulted in increase PGE_2 synthesis and efflux with decrease $PGF_2\alpha$ synthesis and efflux from hepatio tissue of albino rats.

The effect of Ca++ ions on prostaglandins synthesis and release must be put in mined on using drugs which ulter of PG synthesis, efflux or both. An example of these drugs are calcium channel blockers, beta adrenergic receptor stimulator as well as alpha adrenergic receptor stimulator, which are commonly used in the treatment of many cardio vascular diseases. Their use must be put under observation to avoid undesirable effects as a result of increase or decrease prostaglandins formation.

Table (1) : Effect of calcium gluconate on PGE2 and PGF2 α output from isolated hepatic tissue incubated for 2 h. at 37°C.

The tested group	PGE_2 (ng/gm tissue)	$PGF_2\alpha$ ng/gm
1. Control group mean	29.362	33.625
±S. E. M.	5.058	2.251
2. Calcium group Mean	47.451	14.504
±S. E. M.	4.341	2.413
P	0.05*	0.05*

* Highly significant.

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الملخص العربى

أجرى هذا البحث لدراسة تأثير أيونات الكالسيوم على البروستا جلاندين المستخرج من خلايا كبد الفئران البيضاء.

وقد أدت نتائج البحث إلى أنه اضافة أيونات الكالسيوم إلى محلول كربس رينجر فوسفات المحتضن للخلايا بجرعة ٢٩ ميكروجرام لكل جرام من وزن الأنسجة على هيئة جلوكونات الكالسيوم قد ساعد على زيادة كمية البروستا جلاندين E2 المستخرج من خلايا الكبد زيادة ذات دلالة احصائية عالية. كما أنه أدى إلى نقص كمية البرستاجلاندين F2 α المستخرج من خلايا الكبد وهذا النقص ذو دلاله احصائية عالية مما يدل على أن أيونات الكالسيوم لها دور فعال فى عملية تكوين البروستاجلاندينات وتحويل كل من النوعين E2 & F2 α إلى الآخر.