CATIONIC DEPENDENCY OF HIGH AFFINITY PROSTAGLANDIN $F_2\alpha$ RECEPTORS IN BOVINE CORPUS LUTEUM CELL MEMBRANES

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SUMMARY

The specific binding of [^{3}H] Prostaglandin (PG) $F_2\alpha$ to bovine corpus luteum cell membranes prepared in homogenizing buffer containing either 1 mM EDTA (H-EDTA) or 1 mM Ca^{2+} (H-Ca^{2+}) was examined. The membranes prepared in H-EDTA buffer bound less [^{3}H] $PGF_2\alpha$ and had a single class of $PGF_2\alpha$ receptors with an apparent dissociation constant (Kd) of 2.7×10^{-8}M. The addition of Ca^{2+} to these membranes resulted in increased binding with the appearance of new $PGF_2\alpha$ receptors of Kd = 4.3×10^{-9}M. The membranes prepared in H-Ca^{2+} buffer contained two classes of receptors with Kds = 2.9×10^{-9}M and 2.9×10^{-8}M. The removal of Ca^{2+} from these membranes resulted in lower binding as well as a complete disappearance of receptors of Kd = 2.9×10^{-9}M. These results suggest the dependency of high affinity $PGF_2\alpha$ receptors, in bovine corpus luteum cell membranes, on cations.

INTRODUCTION

Recent studies from our laboratory as well as others have demonstrated the presence of specific and high affinity receptors for gonadotropins (1-5), prostaglandin (PG) Es (2, 6-8) and for $PGF_2\alpha$ (8-10) in the cell membranes of bovine corpora lutea. It has been shown that the gonadotropin receptors in these membranes are homogeneous (1,3,4,6) whereas, PGE receptors are heterogeneous (6-8). However, conflicting reports have appeared with regard to $PGF_2\alpha$ receptor heterogeneity (8-10). During the course of our work on $PGF_2\alpha$ receptors, we have observed that exclusion of Ca^{2+} or substitution of Ca^{2+} (1 mM) by EDTA (1 mM) in homogenizing and incubation buffers results in much lower [^{3}H] $PGF_2\alpha$ specific binding (11). The present paper explores the nature of the

decreased binding in the presence of EDTA which led to the findings on cationic dependency of high affinity $PGF_2\alpha$ receptors.

MATERIALS AND METHODS

Unlabeled $PGF_2\alpha$ was generously donated by Dr. John Pike of the Upjohn Co., Kalamazoo, Mich. [^{3}H] $PGF_2\alpha$ (178 Ci/mmole) was purchased from New England Nuclear Corp.

Procedures for checking the purity of [^{3}H] $PGF_2\alpha$, its purification if needed, collection of bovine corpora lutea, isolation of the cell membranes and performance of [^{3}H] $PGF_2\alpha$ binding assays were the same as those described earlier (9, 11-13) (see table and figure legends for additional details). The homogenizing buffer that was used in the isolation of the cell membranes (0.01 M Tris-HCl, pH 7.0 containing 0.25 M sucrose, 1 mM dithiothreitol and 0.1% gelatin) contained either 1 mM EDTA (referred to as H-EDTA) or 1 mM Ca^{2+} (referred to as H-Ca^{2+}). The protein content in an aliquot of the cell membranes was determined by the method of Lowry et al (14) using bovine serum albumin as the standard.

Homogenizing buffer was used in preparing the ion solutions. The pH of all ion solutions was adjusted to about 7.0 before addition to the membranes. The non-specific binding was determined in each experiment by incubating identical amounts of membrane protein and [^{3}H] $PGF_2\alpha$ in the presence of excess unlabeled $PGF_2\alpha$ (2.8×10^{-5}M). The specific binding, which is presented in all the figures, was obtained by subtracting non-specific binding from total binding.

RESULTS

Binding Studies on Membranes Prepared in H-EDTA Buffer - In our previous studies on [^{3}H] $PGF_2\alpha$ receptors (9,11), H-Ca^{2+} buffer was used for isolation of cell membrane fractions from bovine

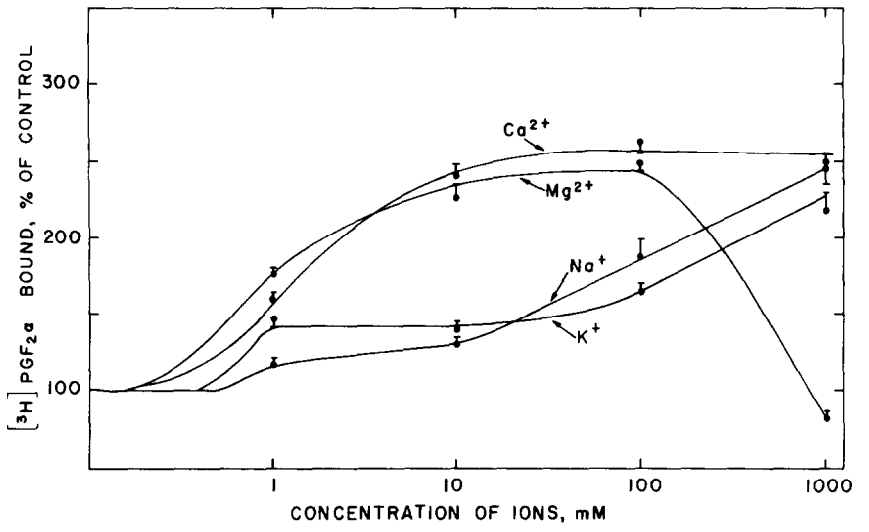

Fig. 1 Cationic restoration of [^{3}H] $PGF_2\alpha$ binding to bovine corpus luteum cell membranes prepared in H-EDTA buffer. Tubes containing 253 µg membrane protein, 3.4 x 10^{-9}M [^{3}H] $PGF_2\alpha$ and increasing concentrations of different cations were incubated for 1 hr at 22°. The amount bound in control tubes (contained none of the ions) was taken as 100%.

corpora lutea. The substitution of Ca^{2+} by EDTA or simple exclusion of Ca^{2+} in the above buffer resulted in decreased [^{3}H] $PGF_2\alpha$ binding to the membranes (11). In the present studies, attempts were made to restore the binding by adding various mono and divalent cations to the membranes prepared in H-EDTA buffer. As shown in Fig. 1, addition of as little as 1 mM Ca^{2+} and Mg^{2+} significantly increased the binding. The maximal restoration was obtained at concentrations of 10 mM Ca^{2+} and Mg^{2+}. A further increase in Ca^{2+} concentration resulted in no further increase in binding. However, a similar increase in Mg^{2+} concentration resulted in a decline in binding back to control levels. The addition of Na^+ and K^+ partially restored the binding, however, they were less effective compared to Ca^{2+} and Mg^{2+} at concentrations below 1000 mM.

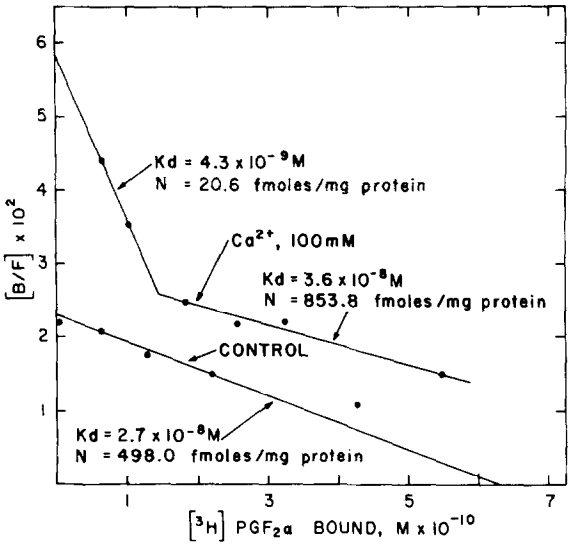

Fig. 2 Scatchard plot analysis of Ca^{2+} induced restoration of [^{3}H] $PGF_{2\alpha}$ binding to bovine corpus luteum cell membranes prepared in H-EDTA buffer. Aliquots of 253 µg membrane protein were incubated for 1 hr at 22° with and without (control) added Ca^{2+} in the presence of increasing concentrations of [^{3}H] $PGF_2\alpha$ (3.4×10^{-10}M to 6.6×10^{-8}M). The apparent dissociation constants (Kd) and the number of receptor sites (N) were calculated from the reciprocal of the slopes and from the x-axis intercepts respectively.

The nature of cation induced restoration of [^{3}H] $PGF_2\alpha$ binding was examined by Scatchard plot analysis (15). As shown in Fig. 2, the [^{3}H] $PGF_2\alpha$ binding to the membranes prepared in H-EDTA buffer was homogeneous indicating the presence of a single set of $PGF_2\alpha$ receptors (Kd = 2.7×10^{-8}M). When maximal restoring concentrations of Ca^{2+} were added to these membranes, [^{3}H] $PGF_2\alpha$ binding became heterogeneous in nature. The new sites had an apparent Kd of 4.3×10^{-9}M. The addition of Ca^{2+} also resulted in a 71% increase in the number of already existing $PGF_2\alpha$ receptors.

Binding Studies on Membranes Prepared in H-Ca^{2+} Buffer - Figure 3 shows that both mono and divalent cations, in concentrations below 100 mM, had very little effect on [^{3}H] $PGF_2\alpha$ binding to the

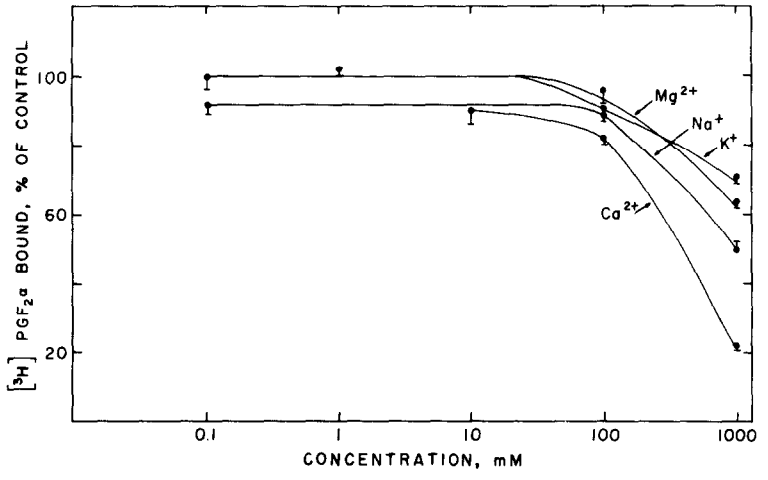

Fig. 3 Effect of cations on [^{3}H] $PGF_2\alpha$ binding to bovine corpus luteum cell membranes prepared in H-Ca^{2+} buffer. Aliquots of 360 µg membrane protein were incubated for 1 hr at 22° with 2.9 x 10^{-9}M [^{3}H] $PGF_2\alpha$ and indicated concentrations of different cations. The amount bound in control tubes was taken as 100%.

membranes. At concentrations above 100 mM, however, these cations caused a moderate to drastic inhibition of binding. Ca^{2+} was the most effective and K^+ was the least effective in inhibiting binding.

In the next experiment, subsequent to the preparation of membranes in H-Ca^{2+} buffer, Ca^{2+} was removed from aliquots of membranes by centrifugation, washing and resuspending in H-EDTA buffer. The [^{3}H] $PGF_2\alpha$ binding to membranes before (control) and after removal of Ca^{2+} was analyzed by Scatchard plot analysis. As shown in Fig. 4, [^{3}H] $PGF_2\alpha$ binding to control membranes was heterogeneous indicating the presence of two sets of receptors (Kds = 2.9 x 10^{-9}M and 2.9 x 10^{-8}M). Following the removal of Ca^{2+} from the membranes, the heterogeneous nature of [^{3}H] $PGF_2\alpha$ binding became homogeneous in nature. The high affinity receptors had disappeared completely and there was a decrease in the number of low affinity receptors.

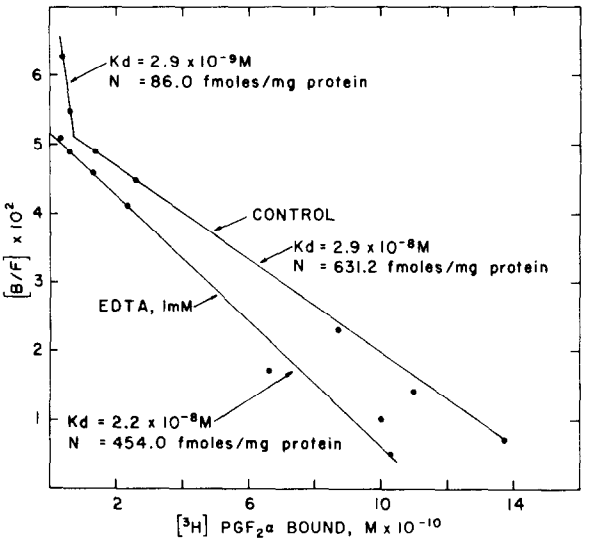

Fig. 4 Scatchard plot analysis of [^{3}H] $PGF_2\alpha$ binding to bovine corpus luteum cell membranes prepared in H-Ca^{2+} (control) buffer and to membranes prepared in the same buffer, subsequently centrifuged, washed and resuspended in H-EDTA buffer. Aliquots of 250 µg membrane protein were incubated for 2 hr at 22° with increasing concentrations of [^{3}H] $PGF_2\alpha$ (5.7×10^{-10}M to 2.0×10^{-7}M).

DISCUSSION

The important feature of these experiments was that the presence or absence of Ca^{2+} in homogenizing and incubation buffers not only influenced the amount of [^{3}H] $PGF_2\alpha$ bound but also the specific group of receptors found in the membrane preparations. Among the two groups of $PGF_2\alpha$ receptors, only high affinity receptors exhibited a dependency on cations. The evidence for cationic dependency of high affinity $PGF_2\alpha$ receptors is two fold: (a) The membranes prepared in H-EDTA buffer or in H-buffer containing no Ca^{2+} (data not shown) lack high affinity receptors and the addition of Ca^{2+} results in their appearance. (b) Membranes prepared in H-Ca^{2+} buffer contain high affinity $PGF_2\alpha$ receptors

which disappear following the removal of Ca^{2+}. Under the above conditions (a and b) low affinity $PGF_2\alpha$ receptors were present but only their numbers were altered.

The Kd (4.3×10^{-9}M) of the newly appeared sites following the addition of Ca^{2+} to the membranes prepared in H-EDTA buffer compares favorably with the Kd (2.9×10^{-9}M) of high affinity receptor sites found in membranes prepared in H-Ca^{2+} buffer. The number of newly appeared sites were, however, lower than those found in membranes prepared in H-Ca^{2+} buffer. The Kd of low affinity receptors (2.9×10^{-8}M) in membranes prepared in H-Ca^{2+} buffer also compares favorably with the Kds (2.2 and 2.7×10^{-8}M) of low affinity receptors found in the same membranes following removal of Ca^{2+} and in the membranes prepared in H-EDTA buffer. The addition of Ca^{2+} to membranes prepared in H-EDTA buffer resulted in a 71% increase while removal of Ca^{2+} from membranes prepared in H-Ca^{2+} buffer resulted in a 28% decrease in the number of low affinity receptors. The loss of high affinity $PGF_2\alpha$ receptors in the absence of cations appears to be at least partly reversible in nature. It is, therefore, reasonable to suggest that high affinity $PGF_2\alpha$ receptors are present perhaps all the time in the membranes but their detection requires the presence of cations in the buffers. Divalent cations appear to be more effective than monovalent cations in this regard.

The exact reasons for the cationic dependency of high affinity $PGF_2\alpha$ receptors is not known. But it must be considered possible in view of the recent observations (16) that cations may bind to sites in membranes. The possible existence of allosteric effects between cation binding sites and $PGF_2\alpha$ receptors may cause conformational changes resulting in the appearance of high affinity receptors. The present communication is only the second

of its kind in demonstrating cationic dependency of high affinity receptors. The first report dealt with similar observations on angiotensin II receptors in adrenal cortex (17). The effective cations in these studies were K^+ and Na^+ (17). The cationic dependency of high affinity $PGF_2\alpha$ receptors must be considered physiological because the ionic strength and divalent cation concentrations in tissues (18,19) are high enough to maintain high affinity $PGF_2\alpha$ receptors.

It has been reported very recently that bovine corpus luteum cell membranes contain a single class of $PGF_2\alpha$ receptors with a Kd of 2.1×10^{-8}M (8) and 5.0×10^{-8}M (10). The above Kds were very close to the Kd of low affinity sites found in membranes prepared in H-Ca^{2+} buffer, the Kd of the sites found in the same membranes following removal of Ca^{2+}, the Kd of sites found in membranes prepared in H-EDTA buffer and the Kd of sites found in membranes prepared in H-buffer containing no Ca^{2+} (data not shown). The buffers used for homogenization and incubation by Kimball and Lauderdale (8) and Powell et al (10) do not contain Ca^{2+}. Therefore, it appears quite likely that the absence of high affinity $PGF_2\alpha$ receptors in their membrane preparations may be related to the lack of Ca^{2+} in the buffers used.

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