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EFFECT OF CALCIUM ON CORTICOSTEROID SECRETION BY ISOLATED FROG INTERRENAL GLAND

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Summary—The direct effect of extracellular calcium concentrations on corticosteroidogenesis has been examined in the frog, using a perifusion system technique. The release of corticosterone and aldosterone in the effluent medium was monitored by specific radioimmunoassays. Increasing concentrations of Ca^{2+} (from 2 to 15 mM) gave rise to a dose-related stimulation of corticosteroid release, whereas the increment of either Na^+ or K^+ concentrations did not modify steroid production. Iterative administration of a moderate concentration of calcium (6 mM) led to a reproducible stimulation of steroid secretion whereas the same dose infused during 6 h induced a transient rise in corticosteroid secretion followed by a plateau. The direct effect of Ca^{2+} on steroidogenesis was confirmed by the dose-dependent stimulation of steroid secretion induced by the calcium ionophore A 23187. Perifusion with a calcium-free medium or blockade of Ca^{2+} channels by 4 mM Co^{2+} both resulted in a significant decrease in steroid production. Conversely, the administration of verapamil (up to 10^{-4} M) did not affect steroidogenesis. These results provide evidence that extracellular calcium ions are required for basal production of corticosteroids in amphibians and that Ca^{2+} influx does not occur through voltage-dependent channels. Since, in the frog, blood Ca^{2+} concentrations vary in a rather large range, these results suggest that circulating Ca^{2+} levels may regulate corticosteroid production in these animals.

INTRODUCTION

It is well known that, in mammals, ACTH is the major corticotropic peptide stimulating the production of glucocorticoids by zona fasciculata cells, whereas zona glomerulosa cells are controlled by various agents including ACTH, angiotensin, serotonin and potassium, which stimulate the biosynthesis of aldosterone [1]. In amphibians, such a differentiation does not exist and the adrenocortical tissue appears to be constituted of only one type of secretory cells which produce similar amounts of corticosterone and aldosterone and are regulated by both ACTH and angiotensin II [2–4]. It is generally accepted that ACTH exerts its action on mammalian adrenocortical cells via an activation of the adenylate–cyclase system [5]. Conversely, several lines of evidence indicate that *c*AMP formation is not involved in the mechanism of action of angiotensin II or potassium on glomerulosa cells [5, 6]. In fact, these two agents are known to be strictly dependent on the presence of calcium salts in the extracellular medium, and it has been proposed that angiotensin II and potassium stimulate adrenal steroidogenesis via an increase in calcium influx [7–9]. Moreover, it has been reported that beef [10] and rat [11] adrenocortical cells could be stimulated by increasing concentrations of external calcium. In amphibians, nothing is known concerning the involvement of calcium in steroid production. It was therefore of interest for us to investigate *in vitro* the effect of manipulating the extracellular calcium concentrations on adrenal steroidogenesis in these animals.

EXPERIMENTAL

Animals

Adult male frogs (*Rana ridibunda* Pallas) originating from Yugoslavia were maintained unfed in glass tanks, under running water. The animals were housed at constant temperature (8 ± 0.5°C) with an established photoperiod of 12 h light/day (light on from 0600–1800 h).

Reagents

Verapamil (Isoptin) was generously provided by Biosedra Laboratories (Malakoff, France). EGTA (Ethyleneglycol-*bis*-(β-aminoethyl ether)-*N,N,N',N'*,-tetraacetic acid), A 23187, Hepes (*N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and lysozyme were purchased from Sigma Chemical Company, Saint-Louis, Missouri. [1,2,6,7-^{3}H]Corti- costerone and [1,2,6,7-^{3}H] aldosterone were purchased from Amersham.

Perifusion system technique

The perifusion technique used in this study has been described in details previously [2]. Briefly, kidneys were removed between 8:00 and 9:00 h from 7 freshly decapitated animals. The interrenal glands were immediately dissected and diced in 1-mm^3 fragments. The explants were pre-incubated for 15 min at

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room temperature in 5 ml of perifusion medium which consisted of Hepes buffer 15 mM containing 2 mg glucose/ml, 112 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$ and 0.3 mg BSA/ml. The perifusion medium was continuously gassed with a 95% O_2–5% CO_2 mixture. Then, the interrenal dice were mixed with preswollen Bio-Gel P_2 (200–400 mesh, Bio-Rad) and packed in a siliconized glass column (0.9 × 12 cm). The interrenal fragments were perifused at a rate of 200 μl/min. The temperature of the column was stabilized at 24°C and the pH of the perifusion medium was maintained at 7.4 throughout the experiment. The tissues were perifused for 2 h before the experimental manipulation began. Column effluent was collected as 5-min fractions during a 12-h period and assayed for corticosterone and aldosterone.

Steroid radioimmunoassays

Corticosterone and aldosterone concentrations were determined by radioimmunoassay as described previously [12, 13]. The specificity of the antibodies was determined using 34 different steroids and related compounds, including the various secretagogues employed. Corticosterone antibodies showed significant cross-reactions with progesterone (19%), 11-deoxycorticosterone (17%) and 21-deoxycortisol (8%) but the cross-reactivity was much lower with all other steroids tested (cross-reactivity for aldosterone and testosterone was 0.63 and 1.8%, respectively). The aldosterone antibodies exhibited very weak cross-reactions (0.005%) with all compounds tested. None of the secretagogues interfered in the corticosterone and aldosterone radioimmunoassays. Preliminary experiments were conducted where effluent medium was submitted to Sephadex LH-20 chromatography prior to the radioimmunoassay. These studies showed that only corticosterone and aldosterone were measured in the assays and that, therefore, these two steroids could be directly assayed without prior extraction of the media. For both assays, the intraassay reproducibility was lower than 2% and the interassay reproducibility was 3%. The sensitivity of the corticosterone and aldosterone assays (5% reduction in antibody-bound counts) was

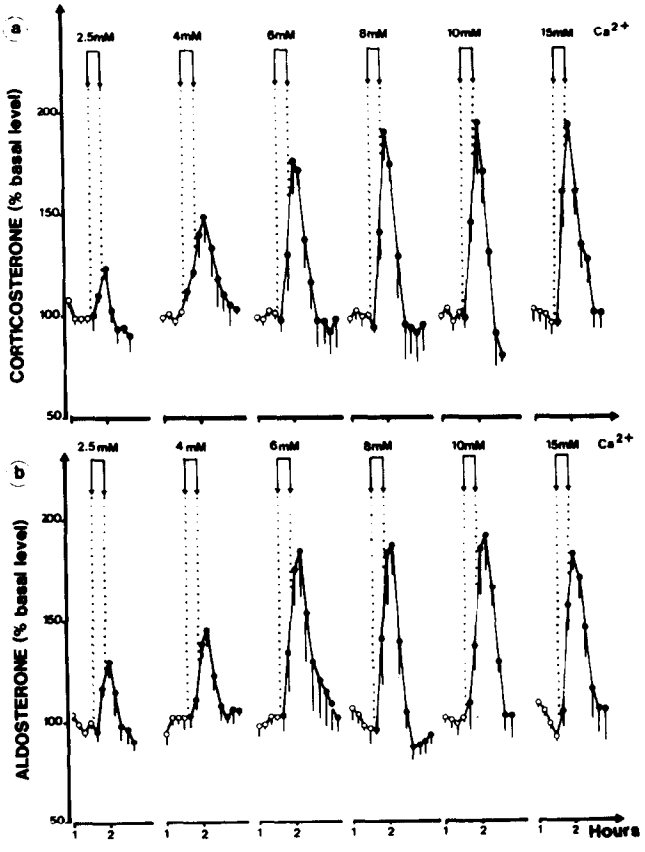

Fig. 1. Effect of increasing doses of Ca^{2+} on corticosterone (a) and aldosterone (b) production. The interrenal fragments were perifused with Hepes buffer containing 2 mM Ca^{2+}. During 20-min periods, graded concentrations of $CaCl_2$ (ranging from 2.5 to 15 mM) were administered. Thus, the lower dose (2.5 mM) corresponds to an increase of 0.5 mM Ca^{2+} above the normal level. Each profile represents the mean secretion pattern of three independent perifusion experiments. The spontaneous level of steroid release, expressed as 100% basal level, was calculated as the mean of eight samples taken just before each dose of $CaCl_2$ (open-circles). For these experiments, the mean secretion rates of corticosterone and aldosterone in basal conditions were 39 ± 3 and 39 ± 12 pg/interrenal/min, respectively.

25 and 5 pg, respectively. Steroid concentrations were calculated from the parameters of standard curves linearized by means of the logit–log transformation.

Calculations

Corticosterone and aldosterone productions were expressed as a percent of the basal values. The basal values were calculated as the mean of 8 samples (40 min) taken just before the infusion of the secretagogues (see legend to Fig. 1 for further details). Where SEM are presented, the results of each perifusion were calculated as the mean profile of corticosteroid production established over three independent experiments. Student's *t*-test for variation analysis was employed to compare the means of different values within the same set of experiments.

RESULTS

Effect of increasing concentrations of calcium on steroid secretion

In resting conditions, steroid secretion by frog interrenal tissue can be maintained up to 14 h. Figure 1 illustrates the levels of corticosterone and aldosterone from interrenal fragments in perifusion. The rate of release of both steroids was quite stable during the whole experiment. Typically, the secretion rate of corticosterone and aldosterone were in the same range (see legend to Fig. 1).

We examined the effect of graded doses of calcium chloride (from 2 to 15 mM) on the release of steroids by perifused interrenal fragments. As shown in Fig. 1, a calcium concentration as low as 2.5 mM, corresponding to an increase of 0.5 mM above the resting level, induced a significant increase in both corticosterone (+15%) and aldosterone (+24%). A 2-fold increase in steroid output was observed with 8 mM. Higher concentrations did not stimulate further the release of corticosteroids. For each dose, the lag period between the beginning of the infusion of high calcium and the onset of the response was 5 min, and the duration of the response was 35–55 min. Secretion patterns of corticosterone and aldosterone were nearly identical.

A series of experiments similar to those represented in Fig. 1a and 1b were conducted with increasing concentrations of potassium and sodium to test whether the action of calcium was specific. As shown in Fig. 2, no detectable change in steroid secretion was observed using graded doses of either Na^+ (114–130 mM) or K^+ (5–15 mM).

Steroid response to repeated doses of calcium

Iterative infusion of high calcium concentration (6 mM) at 100-min intervals led to a reproducible increase in corticosterone and aldosterone secretion (Fig. 3). In addition, the lag period was exactly the same for all stimulations (5 min).

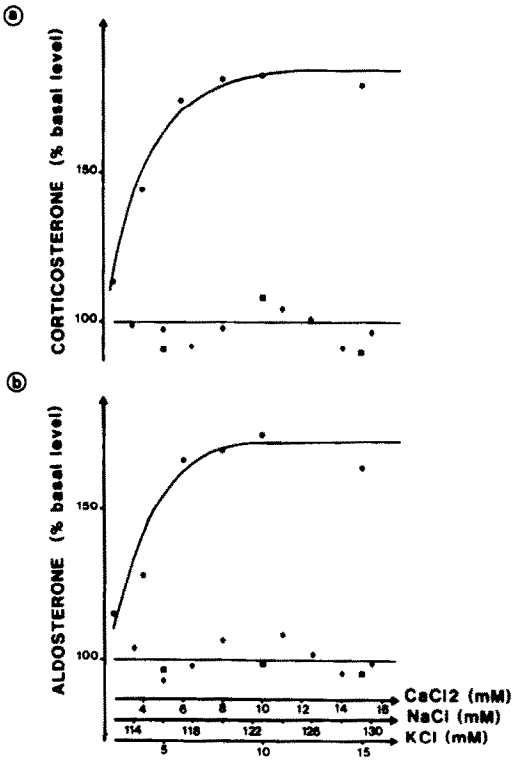

Fig. 2. Comparative effects of increasing concentrations of Ca^{2+} (●--●), Na^+ (◆--◆) and K^+ (■--■) on corticosterone (a) and aldosterone (b) production. Increasing concentrations of $CaCl_2$ (2.5–15 mM), NaCl (114–130 mM) and KCl (5–15 mM) were infused for 20 min at 100-min intervals. All experimental values were calculated from data similar to those represented in Fig. 1. Each point represents the net corticosteroid increase above the basal level in 4 consecutive fractions collected just after salt infusion.

Steroid response to a prolonged infusion of calcium

When the interrenal fragments were perifused in the presence of high Ca^{2+} concentration (6 mM) for 6 h, a biphasic effect was observed (Fig. 4). After a transient rise in corticosteroid release which peaked at 35 min after the onset of high Ca^{2+} infusion, the secretory levels decreased to reach a plateau which was somewhat higher than the spontaneous level. When the 6 mM Ca^{2+} solution was replaced by a 2 mM Ca^{2+} solution, a slight decrease of steroid secretion was observed.

Steroid response to ionophore A 23187

The administration of the calcium ionophore A 23187 at concentrations ranging from 10^{-7} to 3×10^{-6} M gave rise to a dose-dependent stimulation of corticosteroid secretion (Fig. 5). Using the highest concentration of ionophore, a 2.2-fold increase in the secretion rate was observed and the effect was maximum 30–40 min after the onset of the stimulation. These figures were very similar to those measured in the presence of 10 mM Ca^{2+} (Fig. 1). However the duration of the response to A 23187 (120 min for the highest dose) was much longer than that observed with 10 mM Ca^{2+} (60 min).

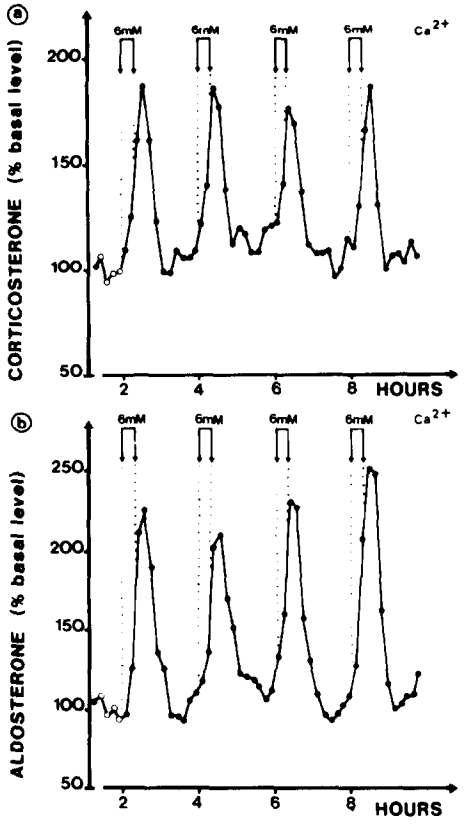

Fig. 3. Effect of repeated doses of high Ca^{2+} on corticosterone (a) and aldosterone (b) production. $CaCl_2$ (6 mM) was infused for 20 min at 100-min intervals. The experiments were carried out in duplicate. The profiles represent the results of one perifusion experiment.

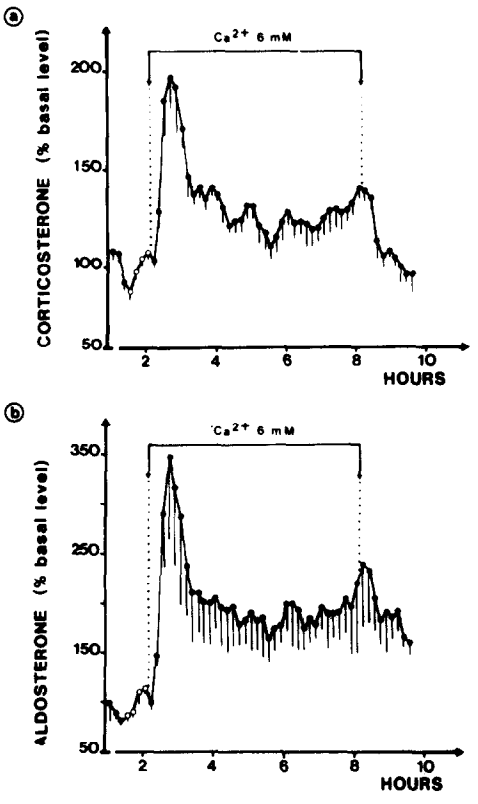

Fig. 4. Effects of a prolonged infusion of high Ca^{2+} on corticosterone (a) and aldosterone (b) production. After a 120-min equilibration period, $CaCl_2$ (6 mM) was administered for 6 h. The glands were then allowed to stabilize for another 90-min period. See legend to Fig. 1 for other designations.

Calcium deprivation

The effect of calcium deprivation on steroid production was investigated using a calcium-free infusion medium supplemented with 0.5 mM EGTA (Fig. 6). In these conditions, a significant inhibition of corticosteroid secretion occurred and reached a maximum (−50%) after 3 h of deprivation. It should be noticed that the effect was not fully overcome when calcium concentration was standardized again. An additionnal phenomenon was observed consequently to each alteration of the medium composition. It consisted in a rapid and transient increase in steroid release which occurred just after the removal of Ca^{2+} from the medium and when the normal concentration of Ca^{2+} was infused again.

Blockade of Ca^{2+}-channels by cobalt

During infusion of 4 mM Co^{2+}, a dramatic inhibition of corticosterone (−88%) and aldosterone (−81%) production was observed within 3 h (Fig. 7). However, a transient stimulation of both steroid secretion (+48% for corticosterone and +61% for aldosterone) occurred just after the onset of Co^{2+} infusion. In addition, the secretion of corticosteroids remained inhibited after withdrawal of Co^{2+} from the medium.

Effect of verapamil on steroid secretion

As shown in Fig. 8, a 40-min infusion of the calcium antagonist verapamil, at doses ranging from 10^{-6} to 10^{-4} M did not significantly modify aldosterone secretion. Similar results were obtained with corticosterone (data not shown).

DISCUSSION

In the present study we have provided evidence that modifications of extracellular concentrations of Ca^{2+} induce dramatic changes of frog adrenal steroidogenesis *in vitro*. Addition of micromolar concentrations of Ca^{2+} stimulated both corticosterone and aldosterone secretion in a dose-dependent manner, indicating that Ca^{2+} acts at an early step in corticosteroidogenesis. The magnitude and the time-course of the steroidogenic response to Ca^{2+} were similar to those elicited by the physiological stimuli ACTH and angiotensin II [4, 14]. In agreement with other studies, which have shown that an increase in Ca^{2+} levels in the extracellular fluid stimulated aldosterone secretion by rat [11, 15, 16] or beef [10] adrenal cortex, our results indicate that the external Ca^{2+} concentration may regulate steroidogenesis. Rather large fluctuations of plasma calcium concen-

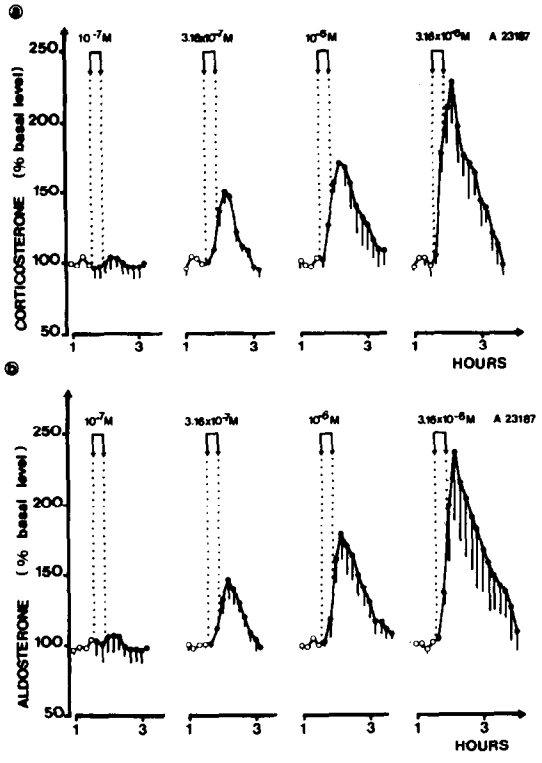

Fig. 5. Effect of increasing doses of calcium ionophore A 23187 on corticosterone (a) and aldosterone (b) production. A 23187 was initially dissolved in dimethyl sulfoxide (DMSO). The final dilution was made up in Hepes buffer so that the concentration of DMSO was 0.2%. Graded doses of A 23187, ranging from 10^{-7} to 3.16×10^{-6} M, were infused for 20 min. A constant concentration of Ca^{2+} (2 mM) was present throughout this experiment.

trations have been reported in intact *Rana pipiens* (1.3–2.15 mM) [17]. In *Rana ridibunda*, we have observed that calcium concentrations in plasma vary from 1.39 to 2.13 mM when the animals are maintained in water for 6 h and from 1.19 to 2.48 mM in terrestrial animals (unpublished results). Since in our study the addition of 0.5 mM calcium enhanced corticosterone and aldosterone secretion, it is conceivable that high blood calcium levels may stimulate directly steroid production in these animals. Such a possibility could be of prime physiological importance in amphibia and may have some pertinent connection with their mode of life. The stimulatory effect of high Ca^{2+} cannot be ascribed to a non-specific ionic alteration of the medium, since the increment of either Na^+ or K^+ concentration did not modify steroid secretion. In addition, the fact that the calcium ionophore A 23187 stimulates corticosteroid secretion by frog adrenal tissue in a dose-dependent manner (as already shown in rat [18], or beef [9] glomerulosa cells) supports the view that Ca^{2+} plays a specific role in adrenocortical secretion. It is interesting to note that a moderate increase in K^+ concentration (5–15 mM) which would stimulate steroidogenesis in mammals [19,20] did not affect steroid secretion in the frog. However, recent studies have shown that depolarizing concentrations of K^+ (24 mM) stimulate and high concentrations of Na^+ (175 mM) inhibit aldosterone secretion in amphibia [21]. The prolonged infusion of 6 mM Ca^{2+} has shown that the rapid stimulation of steroidogenesis was followed by a steady state level slightly higher than the basal secretion rate, suggesting that the activation of extrusion mechanisms of intracellular Ca^{2+} may compensate the initial influx of Ca^{2+}. Such a regulatory system has been recently reviewed by Rasmussen [22].

It should be noted that, in the frog adrenal gland, the chromaffin cells, synthetizing catecholamines and various neuropeptides [23, 24, 25], are in close contact with the adrenocortical cells. Since studies performed in this laboratory have shown that vasoactive intestinal peptide, one of the neuropeptides secreted by the chromaffin tissue, may regulate corticosteroid secretion by a paracrine mechanism [23, 26], the indirect action of Ca^{2+} through chromaffin cells cannot be ruled out.

Studies performed in mammals show that removal of extracellular Ca^{2+} is responsible for a marked decrease of steroid secretion by glomerulosa cells [9, 15, 27]. From a physiological point of view, the frog adrenocortical tissue, which secretes both corticosterone and aldosterone, is homologous to the glomerulosa zona of the mammalian adrenal cortex [4]. The results of the present study show that

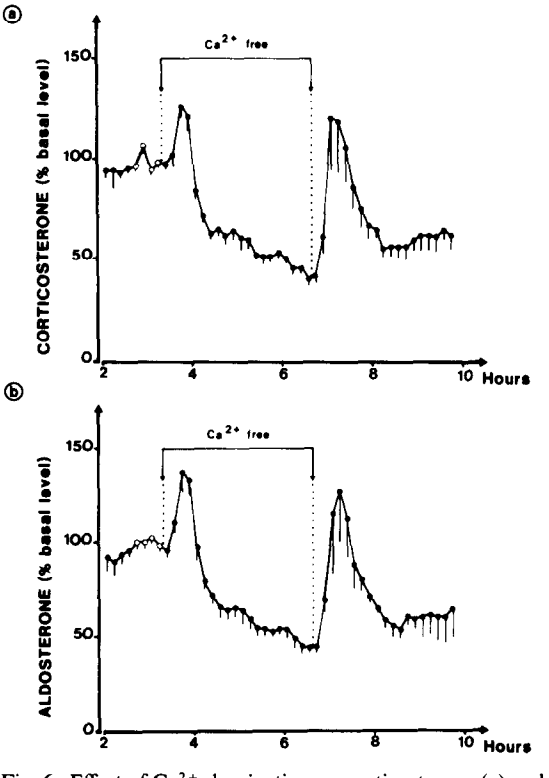

Fig. 6. Effect of Ca^{2+} deprivation on corticosterone (a) and aldosterone (b) production. After a 120-min equilibration period, the interrenal fragments were infused with a Ca^{2+}-free medium supplemented with 0.5 mM EGTA for 3 h. See legend to Fig. 1 for other designations.

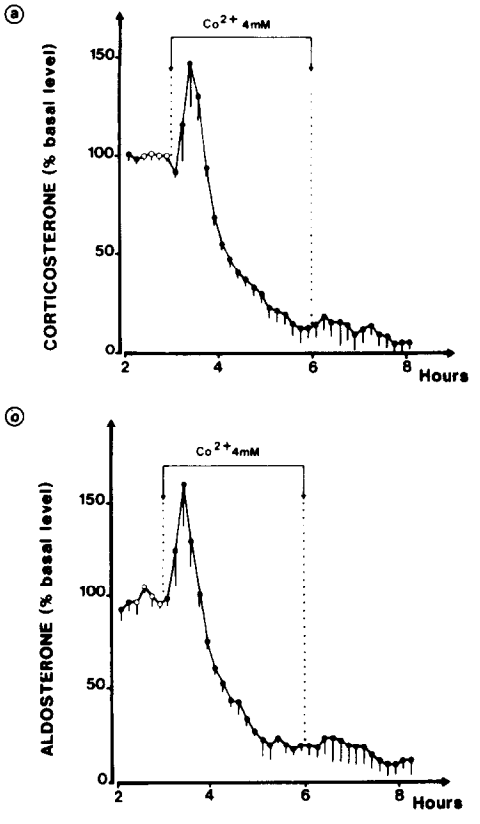

Fig. 7. Effect of Co^{2+} infusion on corticosterone (a) and aldosterone (b) production. $CoCl_2$ (4 mM) was added to the perifusion medium during a 3-h period. A constant concentration of Ca^{2+} (2 mM) was present throughout the experiment. See legend to Fig. 1 for other designations.

infusion of Ca^{2+}-free medium significantly inhibited steroidogenesis from frog adrenal tissue, indicating that calcium uptake plays an important role in the maintenance of spontaneous steroidogenesis. The assumption that calcium influx is required for basal corticosteroid secretion is given strong support by the observation that cobalt, which is known to compete for calcium uptake channels in the plasma membrane [28, 29], dramatically inhibited corticosterone and aldosterone biosynthesis. The prolonged inhibition of steroid production observed after removal of Co^{2+} cannot be accounted for by a non-specific toxic effect of this cation since, cyclic AMP and prostaglandin E_1, were still able to stimulate steroidogenesis after Co^{2+} administration (data not shown). This prolonged inhibitory effect might reflect a high affinity of Co^{2+} for the Ca^{2+} channels. In fact, it has been reported that among other ionic calcium blockers, Co^{2+} dissociates very slowly from Ca^{2+} binding sites [29].

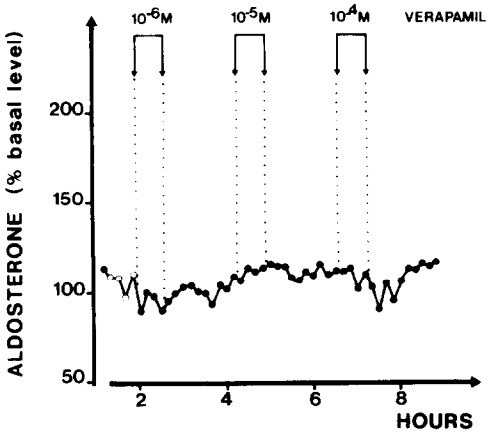

Fig. 8. Effect of verapamil on aldosterone production. Graded doses of verapamil ranging from 10^{-6} to 10^{-4} M were infused for 40 min. A constant concentration of Ca^{2+} (2 mM) was present in the medium. The experiments were carried out in duplicate. The profiles present the results of one perifusion experiment.

It is interesting to note that the perifusion technique is a powerful model to study the time-course effects of calcium antagonists. The biphasic response of the adrenal cells observed in Ca^{2+}-free medium and Co^{2+}- supplemented medium, as revealed in Figs 6 and 7, could not be easily detected by means of static incubation or tissue culture models. The rapid and transitory increase of steroid secretion observed just after Co^{2+} addition, is in harmony with the transient depolarization induced by Co^{2+} administration on mouse zona fasciculata [30]. On the other hand, the important stimulation of steroid secretion observed after restoration of a physiological calcium concentration in the medium might reflect a massive and rapid translocation of calcium through the plasma membrane. Such a stimulant effect of Ca^{2+} reintroduction after a period of Ca^{2+} deprivation has been reported for several other secretory systems, including the adrenal medulla [31] and the intermediate lobe of the pituitary [32].

The lack of effect of verapamil in our model contrasts with several other studies performed in mammals where verapamil has been found to inhibit basal steroidogenesis in rat glomerulosa cells [8, 15, 33]. However, Foster *et al.* [9] have recently shown that methoxyverapamil does not affect the basal production of aldosterone in bovine glomerulosa cells. Since verapamil is known to block Ca^{2+} uptake through voltage-dependent Ca^{2+} channels, it appears that other routes of calcium transit (verapamil independent) must be involved in bovine and frog adrenocortical secretion (i.e. Na^+/Ca^{2+} exchange and Ca^{2+}-bound membrane pool).

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REFERENCES

1. Haning R., Tait S. A. S. and Tait J. F.: *In vitro* effects of ACTH, angiotensins, serotonin and potassium on

steroid output and conversion of corticosterone and aldosterone by isolated adrenal cells. *Endocrinology* **87** (1970) 1147–1167.
2. Leboulenger F., Delarue C., Tonon M. C., Jégou S. and Vaudry H.: *In vitro* study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perifusion system. I. Influence of adrenocorticotropin upon corticosterone release. *Gen. comp. Endocr.* **36** (1978) 327–338.
3. Delarue C., Tonon M. C., Leboulenger F., Jégou S., Leroux P. and Vaudry H.: *In vitro* study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perifusion system. II. Influence of adrenocorticotropin upon aldosterone production. *Gen. comp. Endocr.* **38** (1979) 399–409.
4. Delarue C., Netchitailo P., Leboulenger F., Perroteau I., Escher E. and Vaudry H.: *In vitro* study of frog (*Rana ridibunda* Pallas) interrenal function by use of a simplified perifusion system. VII. Lack of effect of somatostatin on angiotensin-induced corticosteroid production. *Gen. comp. Endocr.* **54** (1984) 333–338.
5. Fujita K., Aguilera G. and Catt K. J.: The role of cyclic AMP in aldosterone production by isolated zona glomerulosa cells. *J. biol. Chem.* **254** (1979) 8567–8574.
6. Shima S., Kawashima Y. and Hirai M.: Studies on cyclic nucleotides in the adrenal gland. VIII. Effects of angiotensin on adenosine 3′,5′-monophosphate and steroidogenesis in the adrenal cortex. *Endocrinology* **103** (1978) 1361–1367.
7. Fakunding J. L., Chow R. and Catt K. J.: The role of calcium in the stimulation of aldosterone production by adrenocorticotropin, angiotensin II and potassium in isolated glomerulosa cells. *Endocrinology* **105** (1979) 327–333.
8. Fakunding J. L. and Catt K. J.: Dependence of aldosterone stimulation in adrenal glomerulosa cells on calcium uptake: effects of lanthanum and verapamil. *Endocrinology* **107** (1980) 1345–1353.
9. Foster R., Lobo M. V., Rasmussen H. and Marusic E. T.: Calcium: its role in the mechanism of action of angiotensin II and potassium in aldosterone production. *Endocrinology* **109** (1981) 2196–2201.
10. Yanagibashi K.: Calcium ion as "second messenger" in corticoidogenic action of ACTH. *Endocr. jap.* **26** (1979) 227–232.
11. Neher R. and Milani A.: Steroidogenesis in isolated adrenal cells: excitation by calcium. *Molec. cell. Endocr.* **9** (1978) 243–253.
12. Leboulenger F., Delarue C., Belanger A., Perroteau I., Netchitailo P., Leroux P., Jegou S., Tonon M. C. and Vaudry H.: Direct radioimmunoassays for plasma corticosterone and aldosterone in frog: I. Validation of the methods and evidence for daily rhythms in a natural environment. *Gen. comp. Endocr.* **46** (1982) 521–532.
13. Leroux P., Delarue C., Leboulenger F., Jegou S., Tonon M. C., Vaillant R., Corvol P. and Vaudry H.: Development and characterization of a radioimmunoassay technique for aldosterone output from perifused frog interrenal tissue. *J. steroid Biochem.* **12** (1980) 473–478.
14. Perroteau I., Netchitailo P., Homo-Delarche F., Delarue C., Lihrmann I., Leboulenger F. and Vaudry H.: Role of exogenous and endogenous prostaglandins in steroidogenesis by isolated frog interrenal gland: Evidence for dissociation in adrenocorticotropin and angiotensin action. *Endocrinology* **115** (1984) 1765–1773.
15. Shima S., Kawashima Y. and Hirai M.: Effects of ACTH and calcium on cyclic AMP production and steroid output by the zona glomerulosa of the adrenal cortex. *Endocr. jap.* **26** (1979) 219–225.
16. Carsia R. V., Segre G. V., Clark I. and Malamed S.: Corticosteroidogenesis *in vitro*: effects of parathyroid hormone, ACTH and calcium. *Proc. Soc. exp. Biol. Med.* **167** (1981) 402–406.
17. Grup R. O.: Parathyroid glands. In *Comparative Endocrinology* (Edited by U. S. von Euler and H. Heller). Academic Press, New York, Vol. I (1963) pp. 325–370.
18. Fakunding J. L. and Catt K. J.: Calcium-dependent regulation of aldosterone production in isolated adrenal glomerulosa cells: effects of the ionophore A 23187. *Endocrinology* **110** (1982) 2006–2010.
19. Tait J. F. and Tait S. A. S.: The effect of changes in potassium concentration on the maximal steroidogenic response of purified zona glomerulosa cells to angiotensin II. *J. steroid Biochem.* **7** (1976) 687–690.
20. Fredlung P., Saltman S., Kondo T., Douglas J. and Catt K. J.: Aldosterone production by isolated glomerulosa cells: modulation of sensitivity to angiotensin II and ACTH by extracellular potassium concentration. *Endocrinology* **100** (1977) 481–486.
21. Maser C., Janssens P. A. and Hanke W.: Stimulation of interrenal secretion in amphibia. I. Direct effects of electrolyte concentration on steroid release. *Gen. comp. Endocr.* **47** (1982) 458–466.
22. Rasmussen H.: Pathways of amplitude and sensitivity modulation in the calcium messenger system. In *Calcium and Cell Function* (Edited by W. Y. Cheung). Academic Press, New York, Vol. IV (1983) pp. 1–61.
23. Leboulenger F., Leroux P., Delarue C., Tonon M. C., Charnay Y., Dubois P. M., Coy D. H. and Vaudry H.: Co-localization of vasoactive intestinal peptide and enkephalins in chromaffin cells of the adrenal gland of amphibia and their action upon corticosteroidogenesis. *Life Sci.* **32** (1983) 375–383.
24. Leboulenger F., Leroux P., Tonon M. C., Vaudry H., Coy D. H. and Pelletier G.: Coexistence of VIP and enkephalins in the adrenal chromaffin granules of the frog. *Neurosci. Lett.* **37** (1983) 221–226.
25. Leboulenger F., Charnay Y., Dubois P. M., Rossier J. and Vaudry H.: Presence of proenkephalin in chromaffin cells of the frog adrenal gland. *Neurochem. Int.* **6** (1984) 773–777.
26. Leboulenger F., Perroteau I., Netchitailo P., Lihrmann I., Leroux P., Delarue C., Coy D. H. and Vaudry H.: Action of vasoactive intestinal peptide (VIP) on amphibian adrenocortical function, *in vitro*. *Peptides* **4** (1984) 299–303.
27. Farese R. V. and Prudente W. J.: Localization of the metabolic processes affected by calcium during corticotropin action. *Biochim. biophys. Acta* **497** (1977) 386–395.
28. Rahwan R. G.: Mechanisms of action of membrane calcium channel blockers and intracellular calcium antagonists. *Med. Res. Rev.* **3** (1983) 21–42.
29. Cavero I. and Spedding M.: "Calcium antagonists": a class of drugs with a bright future. Part I. Cellular calcium homeostasis and calcium as a coupling messenger. *Life Sci.* **33** (1983) 2571–2581.
30. Lymangrover J. R., Matthews E. K. and Saffran M.: Membrane potential changes of mouse adrenal zona fasciculata cells in response to adrenocorticotropin and adenosine 3′,5′-monophosphate. *Endocrinology* **110** (1982) 462–468.
31. Douglas W. W. and Rubin R. P.: The role of calcium in the secretory response of the adrenal medulla to acetylcholine. *J. Physiol. Lond.* **159** (1961) 40–57.
32. Tomiko S. A., Taraskevich P. S. and Douglas W. W.: Potassium-induced secretion of melanocyte-stimulating hormone from isolated pars intermedia cells signals participation of voltage-dependent calcium channels in stimulus-secretion coupling. *Neuroscience* **6** (1981) 2259–2267.
33. Balla T., Hunyady L. and Spät A.: Possible role of calcium uptake and calmodulin in adrenal glomerulosa cells: effects of verapamil and trifluoperazine. *Biochem. Pharmac.* **31** (1982) 1267–1271.