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Activation of 5-Hydroxytryptamine$_4$ Receptors Causes Calcium Influx in Adrenocortical Cells: Involvement of Calcium in 5-Hydroxytryptamine-Induced Steroid Secretion

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SUMMARY

5-Hydroxytryptamine (5-HT) stimulates corticosteroid secretion from adrenal cells through activation of 5-HT$_4$ receptors positively coupled to adenylyl-cyclase. In the present study, we investigated in frog adrenocortical cells the effect of 5-HT$_4$ receptor agonists on cytosolic calcium concentration ([Ca^{2+}]$_i$) and determined the sequence of events associated with 5-HT$_4$ receptor activation. The application of 5-HT or the 5-HT$_4$ receptor agonist zacopride (10^{-8} to 10^{-5} M each) in the vicinity of cultured adrenocortical cells caused a dose-dependent increase in [Ca^{2+}]$_i$. Preincubation of the cells with the selective 5-HT$_4$ receptor antagonist [1-[2-(methylsulfonylamino)ethyl]-4-piperidinyl]methyl-1-methyl-1*H*-indole-3-carboxylate maleate totally blocked the 5-HT-induced stimulation of [Ca^{2+}]$_i$. Chelation of extracellular calcium with ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (10 mM) suppressed the stimulatory effect of 5-HT on [Ca^{2+}]$_i$. Conversely, thapsigargin, an inhibitor of calcium ATPase activity, had no effect on the [Ca^{2+}]$_i$ rise. The calcium influx induced by 5-HT$_4$ receptor agonists was not affected by nifedipine and ω-conotoxin GVIA but was totally blocked by pimozide, a T-type calcium channel antagonist. The [Ca^{2+}]$_i$ response to zacopride was potentiated by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine and markedly reduced by the protein kinase A inhibitor adenosine-3′,5′-cyclic monophosphorothioate. We studied in perifused frog adrenal slices the involvement of [Ca^{2+}]$_i$ rise and cAMP formation in the mechanism of action of 5-HT$_4$ receptor agonists. Zacopride-induced steroidogenesis was significantly reduced in the presence of adenosine-3′,5′-cyclic monophosphorothioate or after suppression of calcium in the perifusion medium. The stimulatory effect of zacopride on corticosteroid secretion was not affected by nifedipine and ω-conotoxin GVIA but was significantly inhibited by pimozide. Taken together, these data indicate that activation of 5-HT$_4$ receptors in adrenocortical cells causes stimulation of adenylyl cyclase and subsequently increases calcium influx through a T-type calcium channel. Both the increased in cAMP formation and the calcium rise are involved in the stimulatory effect of 5-HT on corticosteroid secretion.

The 5-HT$_4$ receptor subtype has been initially characterized in the mouse embryo colliculus (1, 2) and in the guinea pig hippocampus (3). The presence of 5-HT$_4$ receptors has subsequently been demonstrated in other brain areas (4) and in several peripheral organs from various species, such as the rat esophagus (5), guinea pig ileum (6) and colon (7), human atrium (8), and human (9–11) and frog adrenal gland (12–14). These studies have established that 5-HT$_4$ receptors are positively coupled to the adenylyl cyclase system. Further investigations have demonstrated that activation of 5-HT$_4$ receptors can be associated with other transduction mechanisms, including reduction of potassium current in mouse colliculi neurons (15) and stimulation of calcium current in

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; BIMU 8, endo-*N*-(8-methyl-8-azabicyclo-[3.2.1]oct-3-yl)-2,3-dihydro-(1-methyl)ethyl-2-oxo-1*H*-benzimidazole-1-carboxamide HCl; dbcAMP, dibutyryl-cAMP; GR 113808, [1-[2-(methylsulfonylamino)ethyl]-4-piperidinyl]methyl-1-methyl-1*H*-indole-3-carboxylate maleate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; IBMX, 3-isobutyl-1-methylxanthine; PKA, protein kinase A; Rp-cAMPS, adenosine-3′,5′-cyclic monophosphorothioate; [Sar^1, Val^5]AII, [Sar^1,Val^5]angiotensin II; U-73122, 1-[6-[[17β-3-methoxyestra-1,3,5(10)-trien-17-yl]amino]hexyl]-1*H*-pyrrole-2,5-dione; L15, Leibovitz culture medium; zacopride, (*RS*)-4-amino-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamide HCl; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; indo-1/AM, indo-1-acetoxymethylester; [Ca^{2+}]$_i$, intracellular calcium concentration; *R*, 405 nm/480 nm ratio; AUC, areas under the curve; CGRP, calcitonin gene-related peptide.

human atrial myocytes (16). However, the implication of these different pathways in the physiological response induced by 5-HT has not been studied.

The frog adrenal gland represents a valuable model with which to investigate the mechanism of action of 5-hydroxytryptamine on 5-HT_4 receptors. The amphibian adrenal gland is composed of a single population of adrenocortical cells that are homologous to mammalian glomerulosa cells (17) and thus possesses all of the advantages of a cell line without the disadvantages of transformed tumor cells. The pharmacological characteristics of 5-HT_4 receptors in the frog adrenal gland are very similar to those of their mammalian counterpart (14). The effects of 5-HT_4 agonists on second messenger systems can be easily correlated with the final response of the cells by measuring corticosteroid secretion.

In the present study, we investigated the effect of 5-HT_4 receptor activation on calcium mobilization in frog adrenocortical cells in primary culture. The involvement of calcium in the secretory activity of these cells has also been studied with perifused frog adrenal slices.

Materials and Methods

Reagents. L15, collagenase, protease, thapsigargin, nifedipine, pimozide, ω-conotoxin GVIA, 5-HT, dbcAMP, and IBMX were purchased from Sigma Chemical Co. (St. Louis, MO). Rp-cAMPS was obtained from Biolog Life Science Institute (Bremen, Germany). U-73122 was purchased from Biomol Research Laboratories (Plymouth Meeting, PA). Zacopride was a generous gift from Synthélabo Recherche (L.E.R.S., Rueil-Malmaison, France). GR 113808 was provided by Glaxo (Greenford, UK). BIMU 8 was provided by Boehringer Ingelheim (Milan, Italy). The angiotensin II agonist [Sar^1,Val^5]AII was a generous gift from Dr. E. Escher (Sherbrooke, Québec, Canada). Fetal calf serum, kanamycin, and the antibiotic/antimycotic solutions were purchased from GIBCO (Grand Island, NY). Indo-1/AM was obtained from Molecular Probes (Eugene, OR).

Cell culture. Adult male frogs (*Rana ridibunda*; body weight, 40–50 g) were obtained from a commercial source (Couétard, St. Hilaire de Riez, France). The animals were killed by decapitation, and the adrenal glands were dissected free of renal tissue. Adrenal cells were enzymatically dispersed as previously described (18). Briefly, 20 adrenal glands were rinsed three times in L15 medium adjusted to *R. ridibunda* osmolality (L15/water, 1:0.4) and supplemented with 200 mg/l glucose, 63 mg/l $CaCl_2$, and 1% each of the kanamycin and antimycotic/antibiotic solutions (f-L15; pH 7.4). The adrenal cells were then enzymatically dispersed at 24° for 45 min in f-L15 medium containing collagenase type IA (3 mg/ml) and protease from *Bacillus polymyxa* type IX (3 mg/ml). After digestion, the tissue was disaggregated by gentle aspiration through a siliconized Pasteur pipette with a flame-polished tip. The cell suspension was centrifuged (50 × *g*, 5 min) and rinsed three times with f-L15 medium supplemented with 10% heat-inactivated fetal calf serum. The cells were plated onto glass coverslips at a density of 500,000 cells/ml. The cell viability, determined by the Trypan blue exclusion test, was generally >95%. The incubation medium was replaced every day with fresh f-L15 medium. The cells were used after 3–5 days in culture.

Measurement of cytoplasmic Ca^{2+} concentration. To determine the effect of test substances on $[Ca^{2+}]_i$, we studied single adrenocortical cells using microfluorimetry as previously described (18). Briefly, cultured cells plated onto glass coverslips were incubated in darkness (40 min, 24°) with 5 μM indo-1/AM in f-L15 medium. The coverslips were washed with fresh medium and fitted to the stage of a Nikon Diaphot inverted microscope. The microscope was used in the epifluorescence mode with an oil-immersion objective (×100 CF Fluor series). Adrenocortical cells could be easily

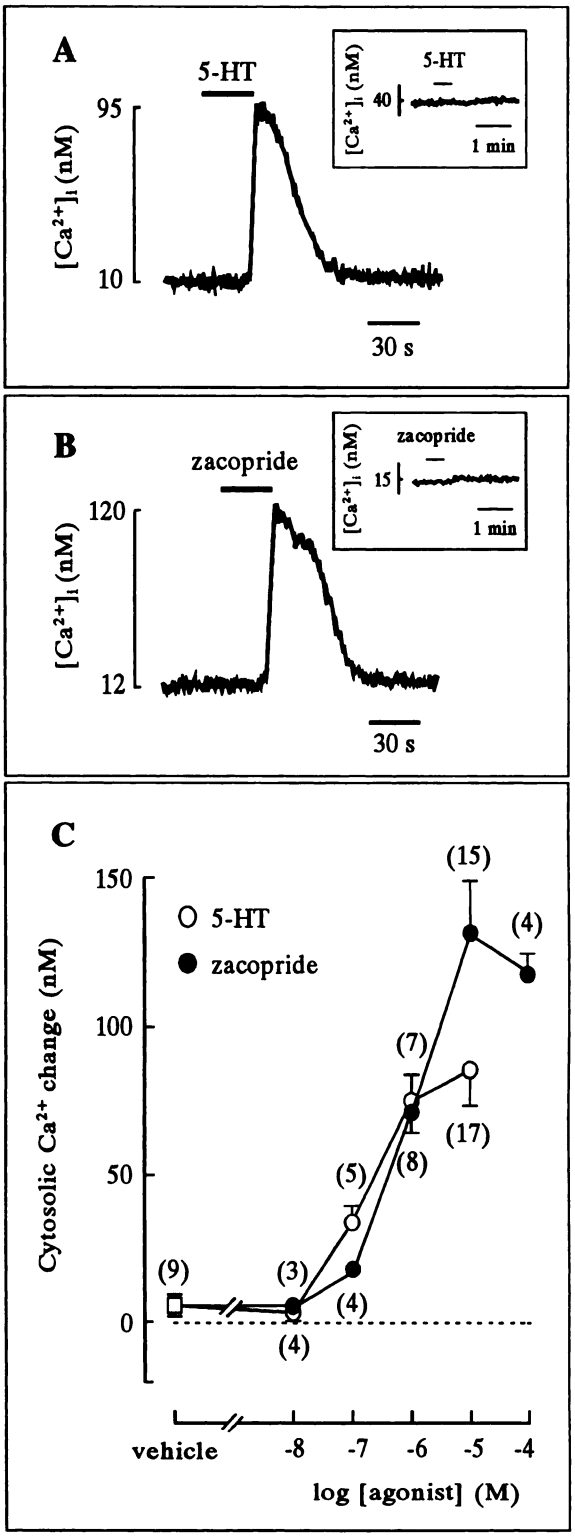

Fig. 1. Effects of 5-HT or zacopride on $[Ca^{2+}]_i$ in cultured adrenocortical cells. A and B, Typical profiles illustrating the effect of a single application (30 sec) of 10^{-5} M 5-HT (A) or 10^{-5} M zacopride (B). *Insets*, neither 5-HT (A) nor zacopride (B) had an effect on $[Ca^{2+}]_i$ in chromaffin cells. *Bars* represent the duration of 5-HT or zacopride administration. C, Dose-response curves showing the effects of graded concentration of 5-HT (○) and zacopride (●) on the amplitude of the $[Ca^{2+}]_i$ response. The data have been calculated from a series of recordings similar to those presented in A and B. The concentrations indicated on the *X*-axis are those contained in the ejection pipette. *Numbers in parentheses*, number of cells studied at each point.

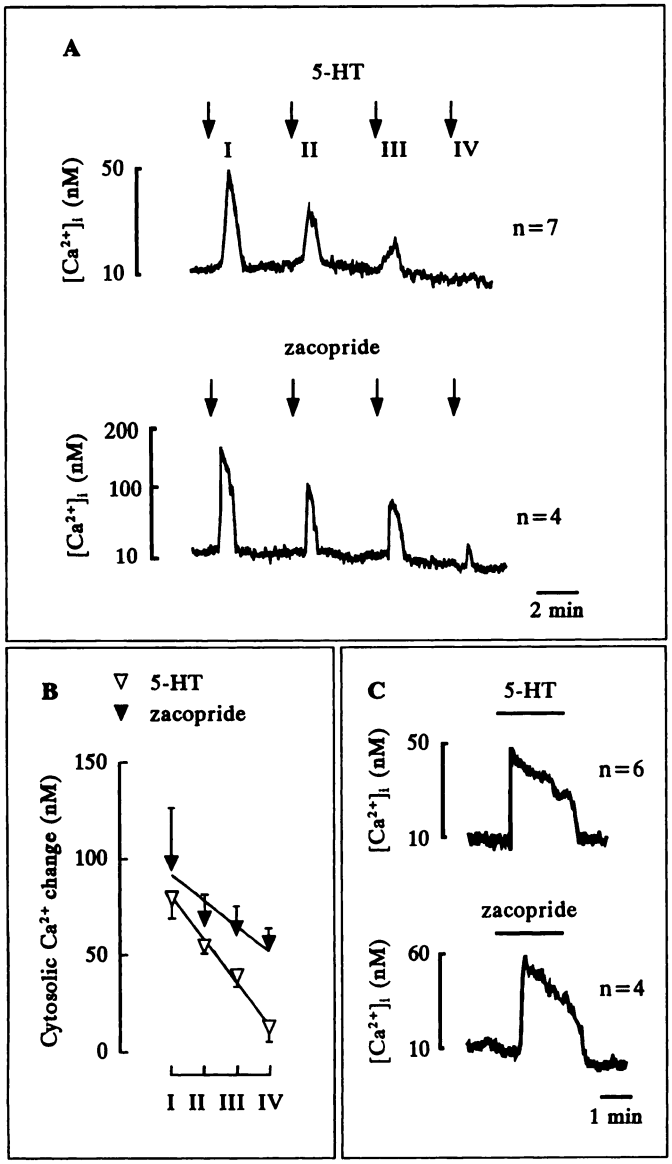

Fig. 2. Effects of repeated or prolonged pulses of 5-HT or zacopride on $[Ca^{2+}]_i$ in cultured adrenocortical cells. A, Typical profiles illustrating the effect of four applications (10 sec) of 10^{-5} M 5-HT (*top*) or 10^{-5} M zacopride (*bottom*) on a single cell. *Arrows*, onset of each pulse of 5-HT or zacopride. B, Plot of the relationship between the number of administrations of 5-HT (▽) or zacopride (▼) and the mean amplitude of the $[Ca^{2+}]_i$ rise. The data have been calculated from a series of recordings similar to those presented in A. C, Typical profiles illustrating the effect of a prolonged administration (2 min) of 10^{-5} M 5-HT (*top*) or 10^{-5} M zacopride (*bottom*) on $[Ca^{2+}]_i$ in cultured adrenocortical cells. *Bars*, duration of 5-HT or zacopride application.

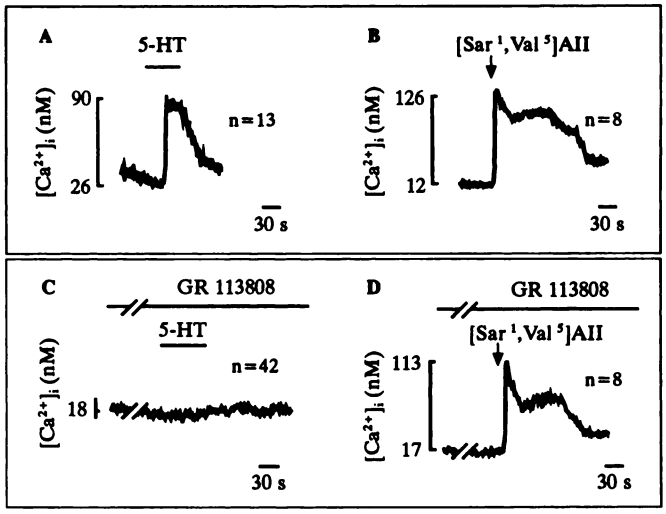

Fig. 3. Effects of the selective 5-HT$_4$ receptor antagonist GR 113808 on 5-HT- or [Sar^1,Val^5]AII-induced $[Ca^{2+}]_i$ rise in cultured adrenocortical cells. A and B, Typical profiles illustrating the effect of a single application (1 min) of 10^{-5} M 5-HT (A) or (5 sec) of 10^{-7} M [Sar^1,Val^5]AII (B). C and D, Typical profiles illustrating the effect of a single application of 10^{-5} M 5-HT (C) or 10^{-7} M [Sar^1,Val^5]AII (D) in the presence of 10^{-7} M GR 113808 in the bath solution. *Bars*, duration of 5-HT application. *Arrows*, onset of 5-HT and [Sar^1,Val^5]AII applications.

distinguished from contaminating chromaffin cells on the basis of their typical morphological features (19). A pressure ejection system was used to deliver test substances in the vicinity of individual cells, with the tip of the ejection glass micropipette placed at a distance of ~100 μm. The fluorescence emission of indo-1, induced by excitation at 355 nm (xenon lamp), was recorded at two wavelengths (405 nm corresponding to the calcium-complexed form and 480 nm corresponding to the free form) by separate photometers (P1, Nikon). The 450 nm/480 nm ratio (R) was determined by using an analogic divider. All three signals (i.e., 405 nm, 480 nm, and R) were continuously recorded with a three-channel voltage recorder (BD 100/101, Kipp & Zonen, Delft, The Netherlands). $[Ca^{2+}]_i$ was calculated according to the equation of Grynkiewicz *et al.* (20):

$$[Ca^{2+}] = KdX\beta\left[\frac{R - R_{min}}{R_{max} - R}\right]$$

where K_d is the dissociation constant for indo-1 (250 nM) (20), β is the ratio of minimal and maximal $[Ca^{2+}]_i$ values at 480 nm, R_{min} is the fluorescence ratio obtained after incubation of cells with f-L15 containing 10 mM EGTA and 10 μM ionomycin for 3 hr, and R_{max} is the fluorescence ratio obtained after incubation of cells with f-L15 containing 10 mM $CaCl_2$ and 10 μM ionomycin for 3 hr. For adrenocortical cells, the average values of R_{min}, R_{max}, and β were 0.163 ± 0.006 (30 cells), 1.720 ± 0.030 (20 cells), and 1.64 (25 cells), respectively. The values of R_{min}, R_{max}, and β for chromaffin cells were 0.140 ± 0.001 (40 cells), 1.640 ± 0.014 (40 cells), and 2.60 (25 cells), respectively. Statistical significance was assessed by two-tailed paired Student's t test, and results were expressed as mean ± standard error.

cAMP measurement. Adrenal glands were dissected, sliced, and preincubated at 24° in 1 ml gassed Ringer's solution. The adrenal tissue was rinsed and incubated for 2 min in the absence or in the presence of various agents. The equivalent of two adrenal glands was used for each incubation tube. The reaction was stopped by the addition of 150 μl of ice-cold 5% perchloric acid. The tissues were then homogenized and centrifuged (10,000 × g for 2 min at 4°). The supernatant was collected, and the pellet was frozen until DNA quantification. The supernatant was neutralized with 1 M $KHCO_3$, diluted in acetate buffer (0.05 M), and stored at −20° until assay. The concentration of cAMP was determined with the use of a commercial kit (Amersham International, UK).

Measurement of corticosteroid secretion. The effect of test substances on steroid release was studied with the use of a perifusion technique as previously described (21). Briefly, adrenal slices were layered into polystyrene columns between several beds of Bio-Gel P2 (Bio-Rad Laboratories, Richmond, CA). The perifusion columns were supplied with Ringer's solution (15 mM HEPES, 112 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 15 mM $NaHCO_3$, supplemented with 0.3 mg/ml bovine serum albumin and 2 mg/ml glucose). The Ringer's solution was continuously gassed with 95% O_2/5% CO_2 and delivered at a constant

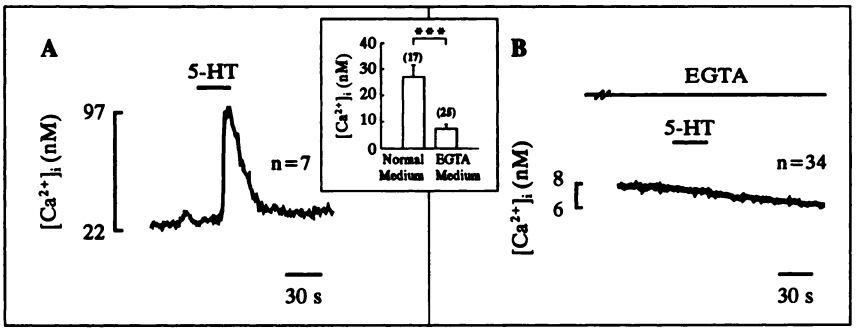

Fig. 4. Effects of EGTA on 5-HT-induced $[Ca^{2+}]_i$ rise in cultured adrenocortical cells. A, Typical profile illustrating the effect of a single application of 5-HT (10^{-5} M; 30 sec) in normal f-L15 medium. B, Effect of 5-HT (10^{-5} M; 30 sec) in the presence of 10 mM EGTA in the bath solution. *Bars*, duration of 5-HT application. *Inset*, effect of addition of EGTA in the bath solution on $[Ca^{2+}]_i$ in resting cells. The number of cells studied for each condition is indicated . ***, $p < 0.001$.

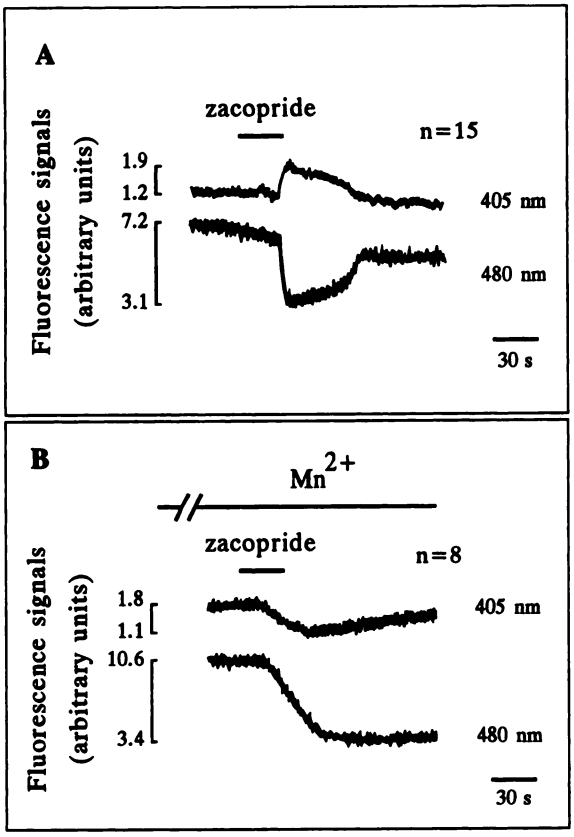

Fig. 5. Effect of Mn^{2+} on the changes of the fluorescence emission signals of indo-1 induced by zacopride in cultured adrenocortical cells. A, Typical profiles recorded at 405 and 480 nm illustrating the effect of a single application zacopride (10^{-5} M; 30 sec) in normal f-L15 medium. B, Effect of zacopride (10^{-5} M; 30 sec) on the 405- and 480-nm fluorescence signals in the presence of 6 mM Mn^{2+} in the bath solution. *Bars*, duration of zacopride application.

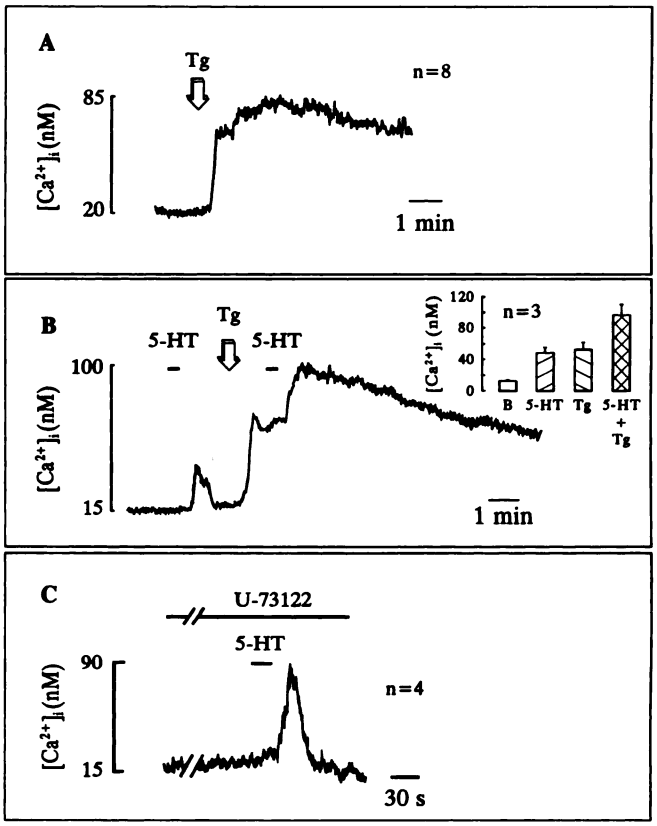

Fig. 6. Effects of thapsigargin or U-73122 on 5-HT-induced $[Ca^{2+}]_i$ rise in cultured adrenocortical cells. A, Typical profile illustrating the effect of a single application (20 sec) of 10^{-5} M thapsigargin (*Tg*; *arrow*) on $[Ca^{2+}]_i$. B, Effect of 5-HT (10^{-5} M; 20 sec) before and after application (20 sec) of 10^{-5} M Tg (*arrow*). *Bars*, duration of 5-HT administration. *Inset*, the effects of 10^{-5} M 5-HT and 10^{-5} M Tg on $[Ca^{2+}]_i$ are strictly additive. C, Effect of 5-HT (10^{-5} M; 20 sec) in cells that had been incubated with the phospholipase C inhibitor U-73122 (10^{-6} M) for 30 min.

flow rate (200 μl/min). The medium was kept at 24° with pH adjusted at 7.35. For calcium-free experiments, $CaCl_2$ was omitted and 0.5 mM EGTA was added to the Ringer's solution. The glands were allowed to stabilize for 2 hr before any test substance was added. Secretagogues were dissolved in the Ringer's solution and infused into the columns at the same flow rate as Ringer's alone with the use of a multichannel peristaltic pump (Desaga, Heidelberg, Germany). Fractions of the effluent perifusate were collected every 5 min, and the tubes were immediately frozen until corticosteroid assays.

Corticosterone and aldosterone concentrations were determined by radioimmunoassays, without prior extraction, in 100–200 μl aliquots from each fraction of effluent perifusate. The characteristics of the radioimmunoassays have been reported previously (22). The assays were sufficiently sensitive to detect 20 pg of corticosterone and 5 pg of aldosterone. For both assays, the intra-assay and inter-assay coefficients of variation were <4% and <10%, respectively.

Each perifusion pattern was established as the mean (± standard error) profile of corticosteroid secretion calculated for at least three independent experiments. The levels of released corticosterone and aldosterone were expressed as percentages of the basal values, calculated as the mean of eight consecutive fractions (40 min) just preceding the infusion of the secretagogues. To compare the net increase in steroid production induced by zacopride in normal and calcium-free media, we calculated the AUCs by using the trapezoidal rule.

Results were expressed as mean ± standard error, and statistical significance was assessed with two-tailed paired Student's *t* test.

Results

Effect of 5-HT and 5-HT$_4$ receptor agonists on [Ca^{2+}]$_i$. The mean [Ca^{2+}]$_i$ in adrenocortical cells under resting conditions was 21 ± 3 nM (51 cells). The cells did not exhibit any spontaneous oscillations. Application of 10^{-5} M 5-HT (30 sec) in the vicinity of adrenocortical cells induced a substantial increase in [Ca^{2+}]$_i$ from 17 ± 2 to 100 ± 8 nM (Fig. 1A; 17 cells). The [Ca^{2+}]$_i$ peaked ~30 sec after the onset of 5-HT administration and returned to base-line within 100 sec. The 5-HT$_4$ receptor agonist zacopride mimicked the effect of 5-HT on [Ca^{2+}]$_i$ (Fig. 1B); application of 10^{-5} M zacopride for 30 sec caused an increase in [Ca^{2+}]$_i$ from 15 ± 2 to 155 ± 18 nM (15 cells). The stimulatory effect of 5-HT on [Ca^{2+}]$_i$ was also mimicked by BIMU 8 (10^{-5} M), another 5-HT$_4$ receptor agonist (data not shown). Neither 5-HT (10^{-5} M) nor zacopride (10^{-5} M) had an effect on [Ca^{2+}]$_i$ in chromaffin cells (Fig. 1, A and B, *insets*; four cells). The stimulatory effects of 5-HT and zacopride on [Ca^{2+}]$_i$ in adrenocortical cells were concentration-dependent (pEC$_{50}$ = 6.50 and 6, respectively), whereas ejection of vehicle in the vicinity of cells (nine cells) has no effect on [Ca^{2+}]$_i$ (Fig. 1C). The proportion of adrenocortical cells responding to 5-HT or to zacopride was also dependent on the concentration of agonist administered, and a significant correlation was found between the concentration of agonist used and the percentage of responding cells (data not shown).

Repeated pulses (10 sec every 4 min) of 5-HT (seven cells) and zacopride (four cells) resulted in a sequential increase in [Ca^{2+}]$_i$, with gradual attenuation of the response (Fig. 2A). Regression analysis of the increase in [Ca^{2+}]$_i$ plotted versus the number of ejections of 5-HT$_4$ receptor agonists yielded a linear regression, with slopes of −22.2 for 5-HT and −13.1 for zacopride (Fig. 2B). Prolonged exposure (2 min) of adrenocortical cells to 5-HT (six cells) or zacopride (four cells) caused a sharp increase followed by a gradual decline in [Ca^{2+}]$_i$ (Fig. 2C).

Effect of a 5-HT$_4$ receptor antagonist on [Ca^{2+}]$_i$. The selective 5-HT$_4$ receptor antagonist GR 113808 was used to test the specificity of the effect of 5-HT on [Ca^{2+}]$_i$ in adrenocortical cells. In the absence of GR 113808, adrenocortical cells responded to 5-HT (10^{-5} M; 13 cells) or to the angiotensin II agonist [Sar^1, Val^5]AII (10^{-7} M; eight cells) by an increase in [Ca^{2+}]$_i$ (Fig. 3, A and B). Administration of GR 113808 alone (10^{-7} M) had no effect on [Ca^{2+}]$_i$ (data not shown). At a concentration of 10^{-8} M, GR 113808 reduced by 45% the number of cells that responded to 10^{-5} M 5-HT. In the presence of GR 113808 (10^{-7} M), the stimulatory effect of 5-HT (10^{-5} M) on [Ca^{2+}]$_i$ was totally abolished (Fig. 3C; 42 cells). In contrast, GR 113808 did not affect the response of the cells to [Sar^1,Val^5]AII (Fig. 3D; eight cells).

Source of calcium involved in 5-HT-induced [Ca^{2+}]$_i$ rise. The addition of 10 mM EGTA to the f-L15 medium reduced the extracellular free calcium concentration from 1.3 mM to 8 nM. After a 30-min incubation with EGTA, the mean [Ca^{2+}]$_i$ in adrenocortical cells was reduced from 27 ± 5 to 8 ± 2 nM (Fig. 4, *inset*) and the stimulatory effect of 5-HT on [Ca^{2+}]$_i$ was totally suppressed (Fig. 4).

The Mn^{2+}-quenching technique was applied to further demonstrate that activation of 5-HT$_4$ receptors is associated with calcium influx. Under normal conditions, the application of 5-HT or zacopride induced an increase in the emission of the fluorescence signal recorded at 405 nm and a concomitant decrease in the signal recorded at 480 nm (Fig. 5A; 15 cells). When adrenocortical cells were incubated in f-L15 medium containing 6 mM Mn^{2+}, the application of zacopride caused a marked reduction in both the 405- and 480-nm signals (Fig. 5B; eight cells), indicating that zacopride actually provoked an influx of Mn^{2+} into the cells.

A brief application of thapsigargin (10^{-5} M; 20 sec) in the vicinity of adrenocortical cells induced a marked and sustained increase in [Ca^{2+}]$_i$ from 15 ± 2 to 86 ± 11 nM (Fig. 6A; eight cells). Fig. 6B shows that thapsigargin did not affect the calcium response to 5-HT (three cells). The stimulatory effects of 5-HT and thapsigargin on [Ca^{2+}]$_i$ were strictly additive (Fig. 6B, *inset*). Incubation of the cells with the phospholipase C inhibitor U-73122 (10^{-6} M) did not affect the calcium response to 5-HT (Fig. 6C; four cells).

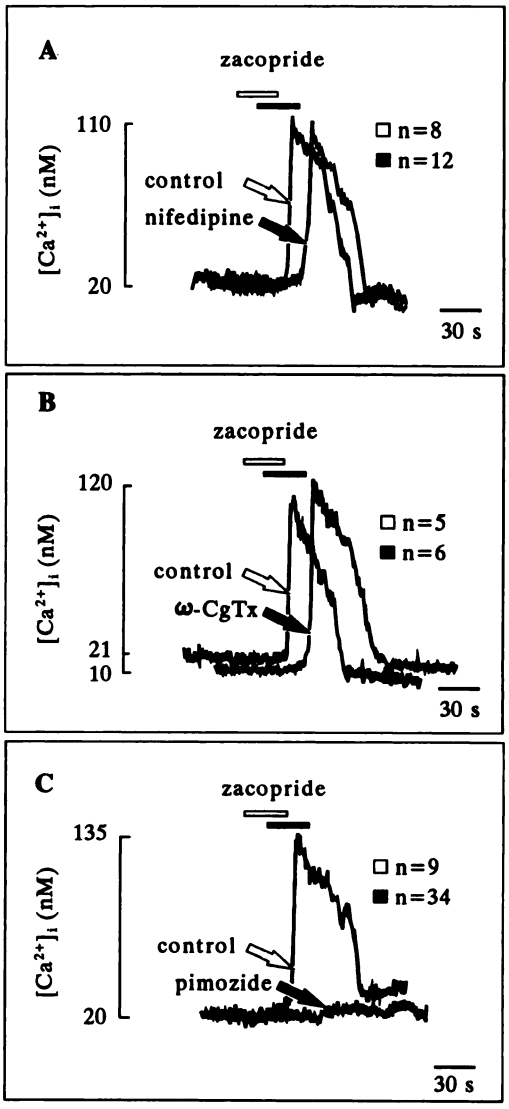

Fig. 7. Effects of various blockers of voltage-sensitive calcium channel on zacopride-induced [Ca^{2+}]$_i$ rise in cultured adrenocortical cells. Typical profiles illustrating the effects of a single application of zacopride (10^{-5} M; 30 sec) in the absence (□) or in the presence (■) of 10^{-5} M nifedipine (A), 10^{-6} M ω-conotoxin GVIA (*ω-CgTx*) (B), or 10^{-5} M pimozide (C). Each calcium channel blocker was added to the bath solution 45 min before zacopride application. *Bars*, duration of zacopride application.

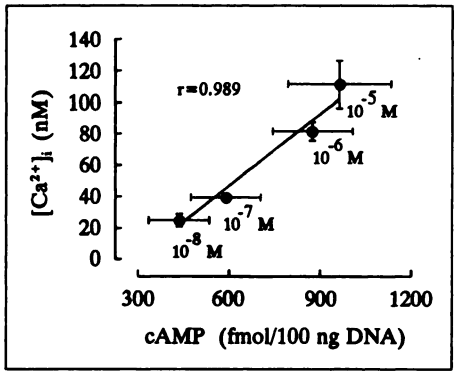

Fig. 8. Correlation between the effects of increasing concentrations of zacopride (10^{-8} to 10^{-5} M) on cAMP formation and $[Ca^{2+}]_i$ rise in adrenocortical cells. Results are expressed as mean ± standard error (3–11 cells). Linear regression analysis was used to determine the correlation coefficient.

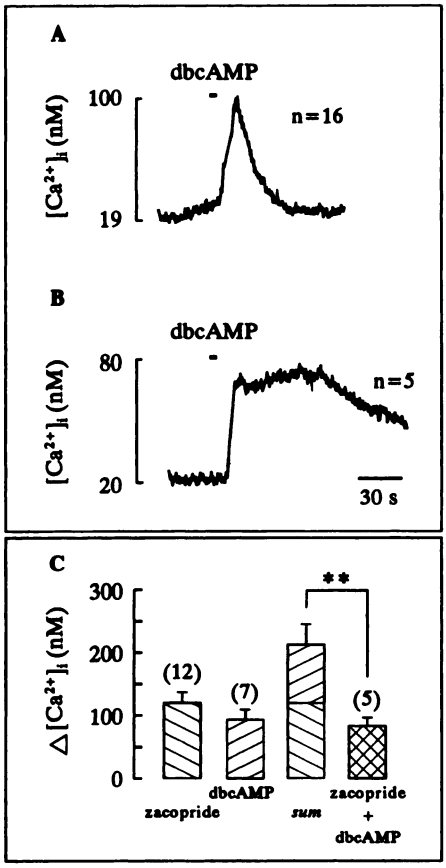

Fig. 9. Effects of dbcAMP on $[Ca^{2+}]_i$ in cultured adrenocortical cells. A and B, Typical profiles illustrating the two types of responses evoked by application of dbcAMP (10^{-4} M; 5 sec). *Bars*, duration of dbcAMP application. C, Comparison of the amplitudes of the $[Ca^{2+}]_i$ rise ($\Delta[Ca^{2+}]_i$) induced by zacopride (10^{-5} M; 20 sec), dbcAMP (10^{-4} M; 20 sec), or concomitant administration of the two drugs. All experimental values are calculated from data similar to those presented in A and B. *Numbers in parentheses*, number of cells studied for each condition. The effect of concomitant administration of zacopride and dbcAMP was significantly lower than the calculated sum of the effects of zacopride and dbcAMP administered separately. **, $p < 0.01$.

Three blockers of voltage-sensitive calcium channels were used to determine the type of channel responsible for calcium influx. Preincubation of the cells with the L-type calcium

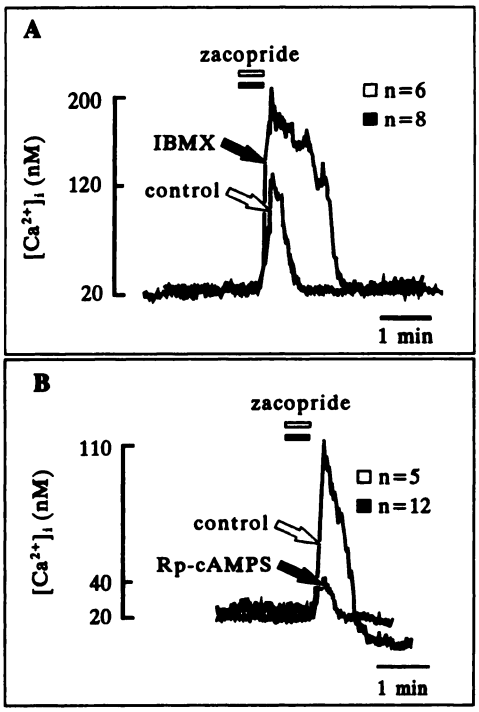

Fig. 10. Effect of IBMX or Rp-cAMPS on zacopride-induced $[Ca^{2+}]_i$ rise in cultured adrenocortical cells. Typical profiles illustrating the effects of a single application of zacopride (10^{-5} M; 30 sec) in the absence (control) or in the presence of 10^{-5} M IBMX (A) or 3×10^{-5} M Rp-cAMPS (B). IBMX and Rp-cAMPS were added to the bath solution 15 min before zacopride application. *Bars*, duration of zacopride application.

channel blocker nifedipine (10^{-5} M; 45 min) did not modify the stimulatory effect of zacopride on $[Ca^{2+}]_i$: $\Delta[Ca^{2+}]_i$ = 120 ± 13 nM in control conditions (eight cells) versus 113 ± 21 nM in the presence of nifedipine (12 cells) (Fig. 7A). Preincubation of the cells with the N-type calcium channel blocker ω-conotoxin GVIA (10^{-6} M; 45 min) caused a 2-fold decrease in the basal $[Ca^{2+}]_i$, from 19 ± 2 nM (20 cells) to 8 ± 2 nM (20 cells). However, the $[Ca^{2+}]_i$ response to zacopride was not affected by ω-conotoxin GVIA: $\Delta[Ca^{2+}]_i$ = 140 ± 15 nM in control conditions (five cells) versus 150 ± 26 nM in the presence of ω-conotoxin GVIA (six cells) (Fig. 7B). In contrast, pimozide (10^{-6} M), which has been shown to inhibit T-type calcium channels (23), significantly reduced ($p < 0.05$) the calcium response to 10^{-5} M zacopride: $\Delta[Ca^{2+}]_i$ = 118 ± 32 nM in control conditions (seven cells) versus 35.5 ± 7 nM in the presence of pimozide (four cells). Incubation of the cells with 10^{-5} M pimozide totally abolished the stimulatory effect of zacopride on $[Ca^{2+}]_i$ (Fig. 7C; 34 cells).

Involvement of a cAMP-dependent pathway in the $[Ca^{2+}]_i$ increase induced by activation of 5-HT$_4$ receptors. Administration of graded doses of zacopride to frog adrenal cells induced a dose-dependent stimulation of cAMP formation. A strict correlation was observed between the stimulatory effects of zacopride on cAMP production and the $[Ca^{2+}]_i$ increase (Fig. 8).

The stimulatory action of 5-HT and zacopride on $[Ca^{2+}]_i$ was mimicked by dbcAMP (10^{-4} M), a permeant analogue of cAMP (Fig. 9). In the majority of the cells (16 of 21), dbcAMP only induced a transient increase in $[Ca^{2+}]_i$ (Fig. 9A). In a few cells (5 of 21 cells), dbcAMP caused a sustained elevation

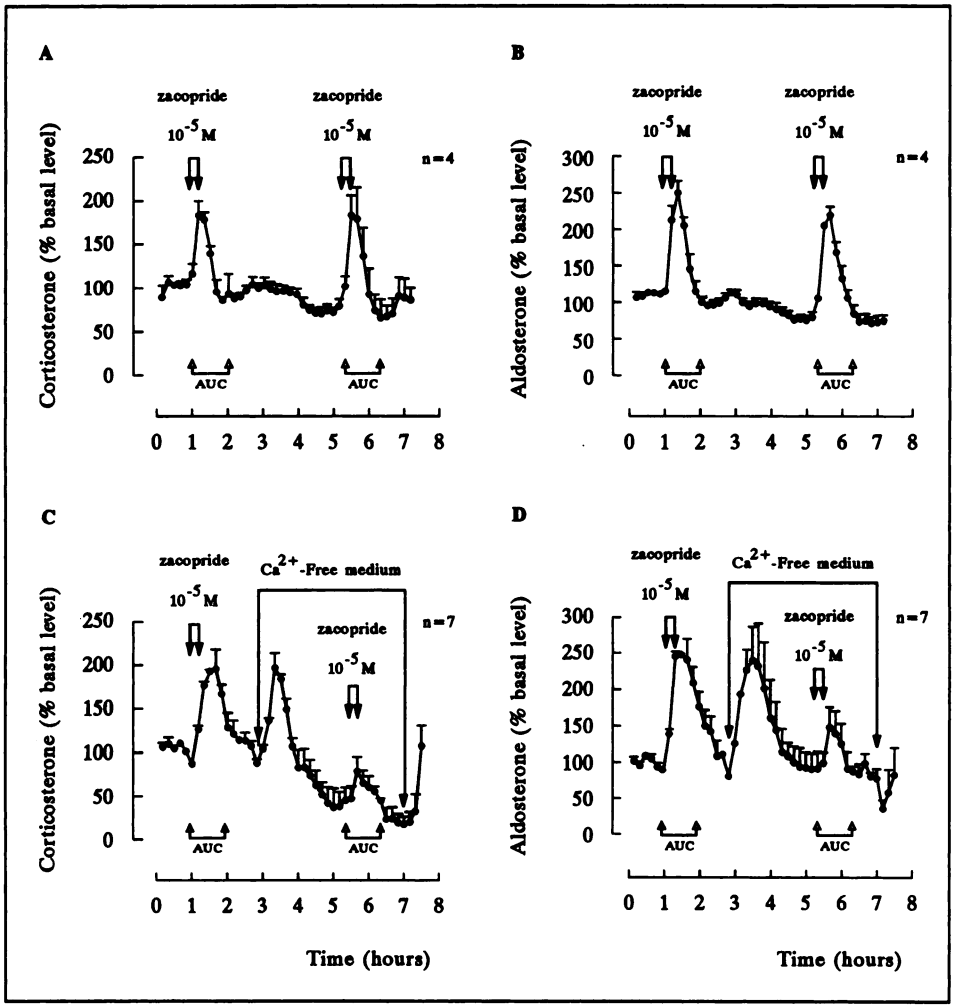

Fig. 11. Effect of calcium-free medium on zacopride-induced steroid secretion by perifused adrenal slices. A and B, Effects of two pulses of zacopride (10^{-5} M; 20 min each) in normal medium (2 mM Ca^{2+}) on corticosterone (A) and aldosterone (B) secretion. The mean secretory rates of corticosterone and aldosterone under basal conditions (100% basal level) were 33.4 ± 3.6 and 16.1 ± 1.8 pg/adrenal gland/min, respectively. C and D, Effects of zacopride (10^{-5} M) in calcium-free medium on corticosterone (C) and aldosterone (D) secretion. A first pulse of zacopride (10^{-5} M) was administered in normal medium for 20 min, and the adrenal tissue was allowed to stabilize for 100 min. Then, the adrenal slices were perifused for 250 min with calcium-free medium supplemented with 0.5 mM EGTA. During infusion of the calcium-free solution, a second pulse of zacopride (10^{-5} M) was administered for 20 min. The mean secretion rates of corticosterone and aldosterone under basal conditions (100% basal level) were 28.5 ± 4.1 and 10.9 ± 1.8 pg/adrenal gland/min, respectively. *Open arrows*, limits of the peaks that were used to calculate the AUCs.

in $[Ca^{2+}]_i$ (Fig. 9B). Concomitant administration of zacopride (10^{-5} M) and dbcAMP (10^{-4} M) did not produce additive effects on $[Ca^{2+}]_i$ (Fig. 9C).

Preincubation of adrenocortical cells with the phosphodiesterase inhibitor IBMX (10^{-5} M) caused an increase in both the amplitude [from 130 ± 12 nM in control conditions (six cells) to 183 ± 7 nM in the presence of IBMX (eight cells) ($p < 0.01$)] and duration [from 68 ± 10 sec in control conditions (six cells) to 100 ± 7 sec in the presence of IBMX (eight cells) ($p < 0.05$)] of the $[Ca^{2+}]_i$ rise induced by zacopride (Fig. 10A). Conversely, preincubation of the cells with the PKA inhibitor Rp-cAMPS (3×10^{-5} M) provoked a marked decrease [from 120 ± 15 nM in control conditions (five cells) to 35 ± 10 nM in the presence of Rp-cAMPS (12 cells) ($p < 0.01$)] of the calcium response to zacopride (Fig. 10B).

Involvement of Ca^{2+} in steroid secretion evoked by activation of 5-HT$_4$ receptors. As previously reported (13, 14), administration of zacopride (10^{-5} M; 20 min) to perifused frog adrenal slices provoked a significant increase in corticosteroid release. In normal Ringer's solution, administration of two pulses of zacopride at a 4-hr interval induced a reproducible stimulation of corticosterone (Fig. 11A, four experiments) and aldosterone (Fig. 11B; four experiments) secretion. Suppression of Ca^{2+} in the perifusion medium caused a massive and transient increase in steroid release (Fig. 11, C and D). During prolonged infusion of Ca^{2+}-free medium, the amplitude of the stimulatory effect of zacopride on corticosterone (Fig. 11C; seven experiments) and aldosterone (Fig. 11D; seven experiments) secretion was markedly attenuated. In calcium-free conditions, the AUCs were significantly reduced ($p < 0.05$) compared with both external (Fig. 11, A and B) and internal (Fig. 11, C and D) controls. As a control, we have investigated the effect of CGRP, which is another potent corticotropic factor in frog (24), on corticosteroid output under the same conditions. It was observed that the stimulatory effect of CGRP on corticosterone and aldosterone secretion was not impaired during the infusion of Ca^{2+}-free medium (data not shown).

Administration of Rp-cAMPS (3×10^{-5} M) to perifused frog adrenal slices significantly attenuated ($p < 0.02$) the stimulatory effect of zacopride on corticosterone (Fig. 12A; four experiments) and aldosterone (Fig. 12B; four experiments) secretion. Administration of Rp-cAMPS in calcium-free conditions totally blocked the secretory response evoked by zacopride (Fig. 12, C and D; three experiments). In contrast, administration of U-73122 (10^{-6} M) did not modify the stimulatory effect of zacopride on corticosterone (Fig. 13A) and aldosterone (Fig. 13B) secretion, whereas at the same concentration, U-73122 totally blocked the response of adrenal cells to ranakinin (25), a novel tachykinin that stimulates corticosteroid secretion in the frog (26).

Administration of nifedipine (10^{-5} M) did not affect the

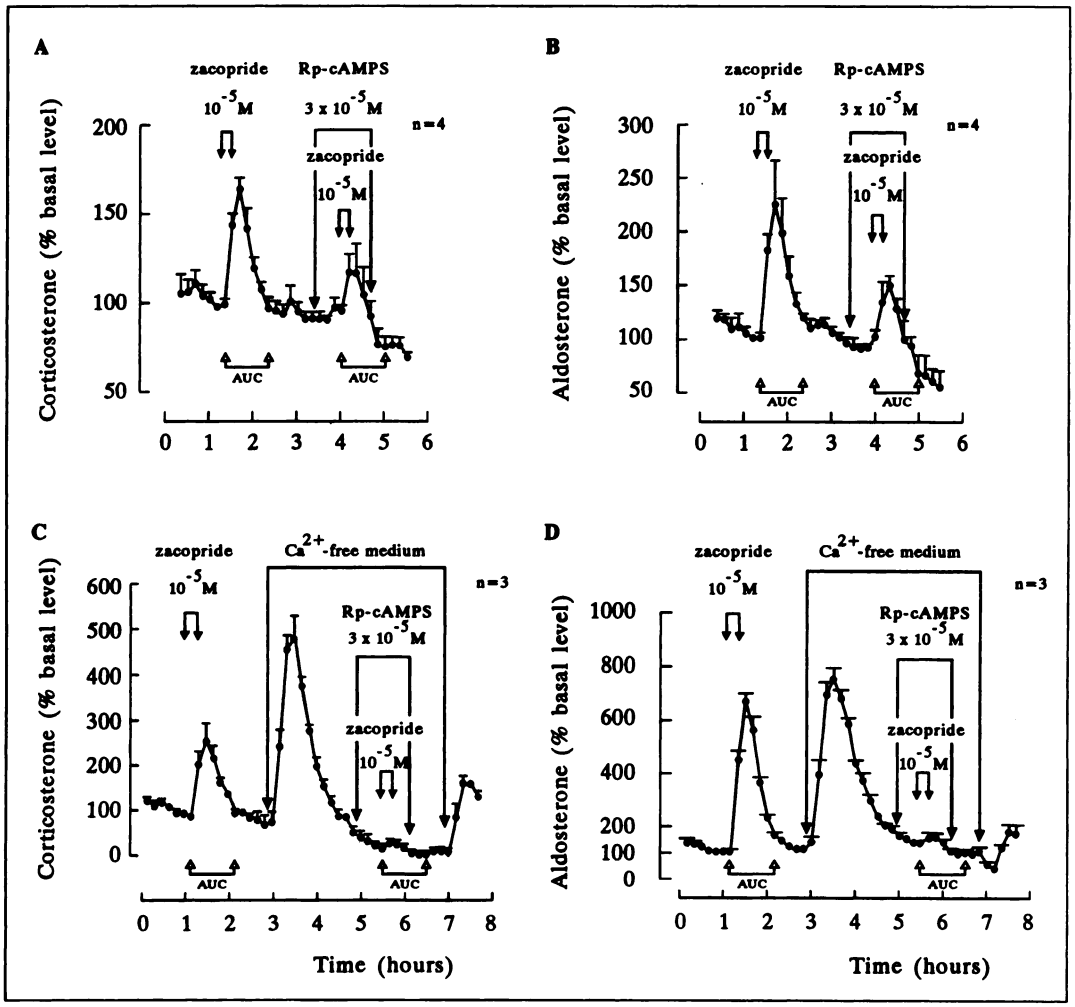

Fig. 12. Effect of Rp-cAMPS, in the presence or absence of calcium, on zacopride-induced steroid secretion by perifused adrenal slices. A and B, Effects of two pulses of zacopride (10^{-5} M; 20 min each) in normal conditions and during prolonged infusion of 3×10^{-5} M Rp-cAMPS on corticosterone (A) and aldosterone (B) secretion. The mean secretory rates of corticosterone and aldosterone under basal conditions (100% basal level) were 56.1 ± 4.9 and 19.1 ± 3.8 pg/adrenal gland/min, respectively. C and D, Effects of two pulses of zacopride (10^{-5} M; 20 min each) in normal conditions and during prolonged infusion of 3×10^{-5} M Rp-cAMPS in calcium-free conditions on corticosterone (C) and aldosterone (D) secretion. The mean secretory rates of corticosterone and aldosterone under basal conditions (100% basal level) were 33.8 ± 7.4 and 10.4 ± 1.6 pg/adrenal gland/min, respectively. (See legend to Fig. 11 for other designations.)

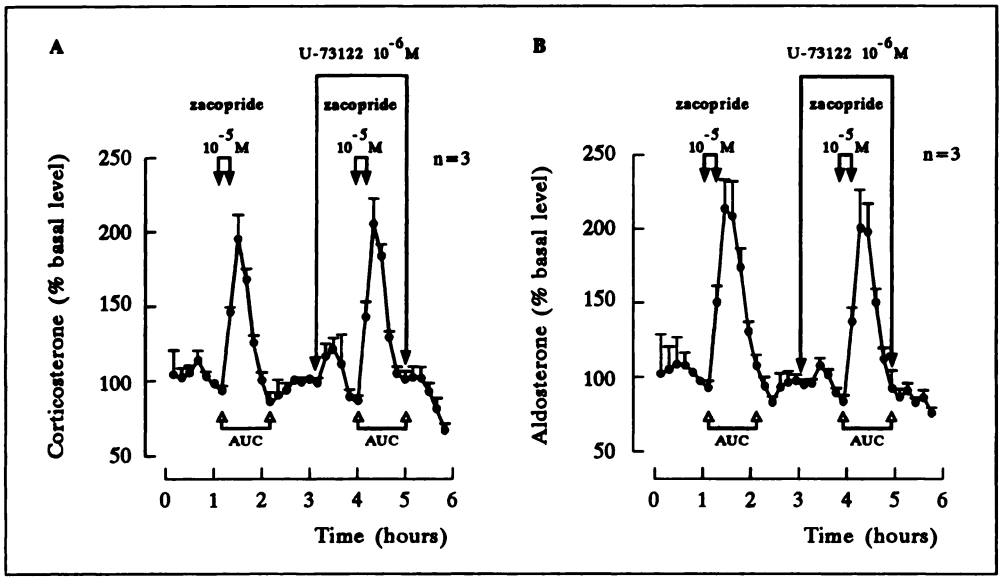

Fig. 13. Effect of the phospholipase C antagonist U-73122 on zacopride-induced steroid secretion by perifused adrenal slices, and effect of two pulses of zacopride (10^{-5} M; 20 min each) under basal conditions and during prolonged infusion of 10^{-6} M U-73122 on corticosterone (A) and aldosterone (B) secretion. The mean secretion rates of corticosterone and aldosterone under basal conditions (100% basal level) were 41.5 ± 5.8 and 29.4 ± 3.4 pg/adrenal gland/min, respectively. (See legend to Fig. 11 for other designations.)

basal secretion of corticosteroids and did not significantly alter the stimulatory effect of zacopride on corticosterone (Fig. 14A; six experiments) and aldosterone (Fig. 14B; six experiments) secretion. At a concentration of 10^{-6} M, ω-conotoxin GVIA caused a slight and transient increase in steroid release, followed by a 43% inhibition of corticosterone secretion (Fig. 14C) and a 41% inhibition of aldosterone secretion (Fig. 14D). During prolonged administration of ω-conotoxin GVIA, the secretory response of the adrenal tissue to zacopride was not significantly modified (Fig. 14, C and D). In contrast, pimozide (10^{-6} M) induced 52 ± 8% and 66 ± 4% inhibition of zacopride-evoked corticosterone and aldosterone

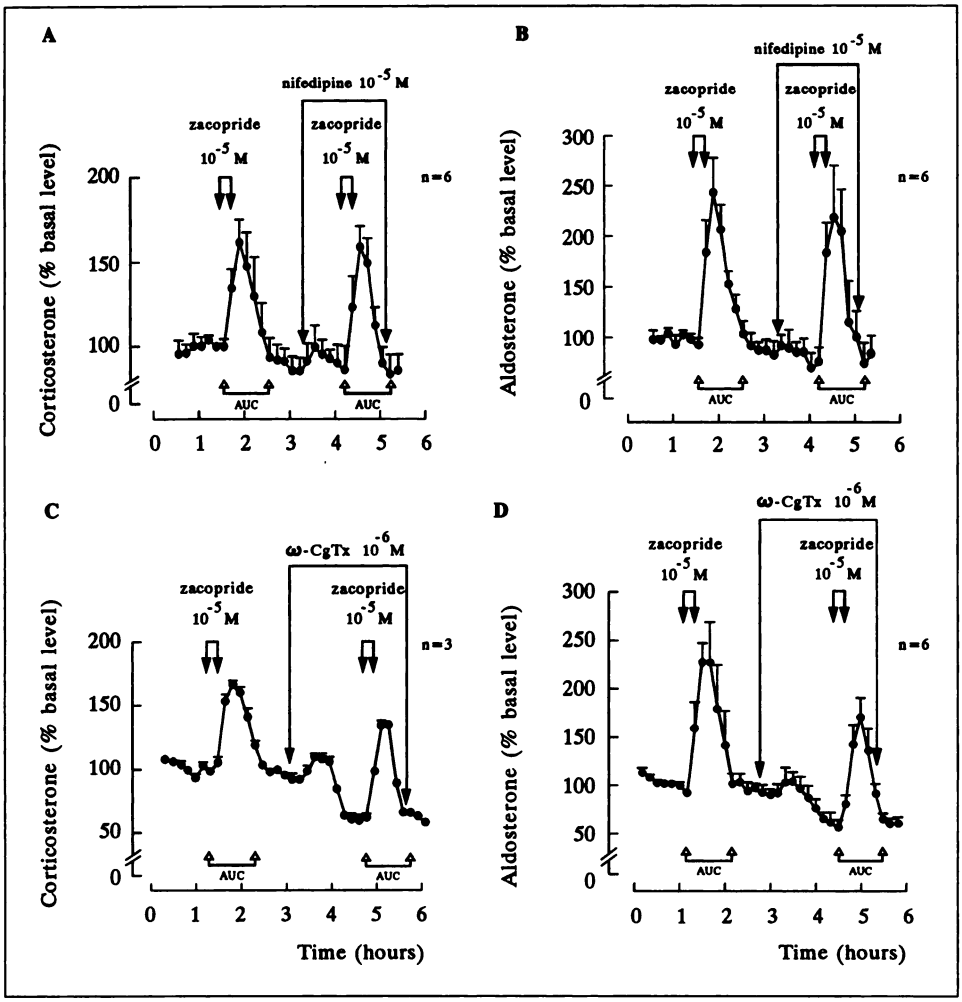

Fig. 14. Effects of blockers of the L- and N-types of voltage-sensitive calcium channels on zacopride-induced steroid secretion by perifused adrenal slices. A and B, Effect of two pulses of zacopride (10^{-5} M; 20 min each) under basal conditions and during prolonged infusion of 10^{-5} M nifedipine on corticosterone (A) and aldosterone (B) secretion. The mean secretion rates of corticosterone and aldosterone under basal conditions (100% basal level) were 42.3 ± 2.8 and 24.4 ± 2.8 pg/adrenal gland/min, respectively. C and D, Effect of two pulses of zacopride (10^{-5} M; 20 min each) under basal conditions and during prolonged infusion of 10^{-6} M ω-conotoxin GVIA (*ω-CgTx*) on corticosterone (C) and aldosterone (D) secretion. The mean secretion rates of corticosterone and aldosterone under basal conditions (100% basal level) were 56.9 ± 7.6 and 24.9 ± 4.6 pg/adrenal gland/min, respectively. (See legend to Fig. 11 for other designations.)

secretion, respectively (Fig. 15, A and B; seven experiments). As a control, the stimulatory effect of CGRP on corticosterone and aldosterone secretion was not significantly altered during infusion of 10^{-6} M pimozide (data not shown). A series of experiments similar to those presented in Fig. 15, A and B, was conducted with different concentrations of pimozide. Pimozide induced a dose-dependent inhibition of the stimulatory effect of zacopride on corticosterone (Fig. 15C) and aldosterone (Fig. 15D) secretion. Two series of control experiments showed that pimozide did not directly interact with 5-HT$_4$ receptors. (i) The stimulatory effect of zacopride on cAMP formation by frog adrenal slices was totally blocked by 10^{-8} M GR 113808 but was not inhibited by 2.5×10^{-5} M pimozide (Fig. 16). (ii) For doses ranging from 10^{-11} to 10^{-5} M, pimozide had no effect on [^{3}H]GR 113808 binding on a membrane-enriched preparation of mouse embryo colliculi, whereas the binding was totally displaced by 10^{-8} M GR 113808 (data not shown).

Discussion

Previous studies have demonstrated that in human and frog adrenal gland, the stimulatory effect of 5-HT can be accounted for by activation of 5-HT$_4$ receptors positively coupled to adenylyl cyclase (9–14). The adrenal cortex, which has a relatively simple organization, offers a convenient experimental model with which to study the transduction mechanisms associated with 5-HT$_4$ receptor activation. An additional advantage of this model is that it makes it possible to correlate the effects on second messenger systems with the final response (i.e., the secretory activity) of the cells. In the present study, we demonstrated that in frog adrenocortical cells, 5-HT$_4$ receptor agonists cause an elevation in $[Ca^{2+}]_i$ that can be ascribed only to calcium influx. This increase in $[Ca^{2+}]_i$ appears to be involved in the stimulatory effect of 5-HT on corticosteroid secretion.

The application of 5-HT in the vicinity of adrenocortical cells induced a dose-dependent increase in $[Ca^{2+}]_i$. The pEC_{50} value (6.50) was very similar to that previously reported for 5-HT-induced corticosterone (6.58) and aldosterone (6.80) secretion (14). Several lines of evidence indicate that the effect of 5-HT on $[Ca^{2+}]_i$ was mediated through activation of 5-HT$_4$ receptors. First, two 5-HT$_4$ receptor agonists (the benzamide derivative zacopride and the azabicycloalkyl benzimidazolone derivative BIMU 8) mimicked the stimulatory action of 5-HT on $[Ca^{2+}]_i$. Both zacopride and BIMU 8 evoked a dose-dependent increase in $[Ca^{2+}]_i$ in very much the same way as 5-HT. The fact that zacopride was more efficient but less potent than 5-HT in stimulating $[Ca^{2+}]_i$ is consistent with previous observations on adenyl cyclase activity in colliculi neurons (2) and corticosteroid secretion from adrenocortical cells (13, 14). Second, the selective 5-HT$_4$ receptor antagonist GR 113808 (27, 28) totally

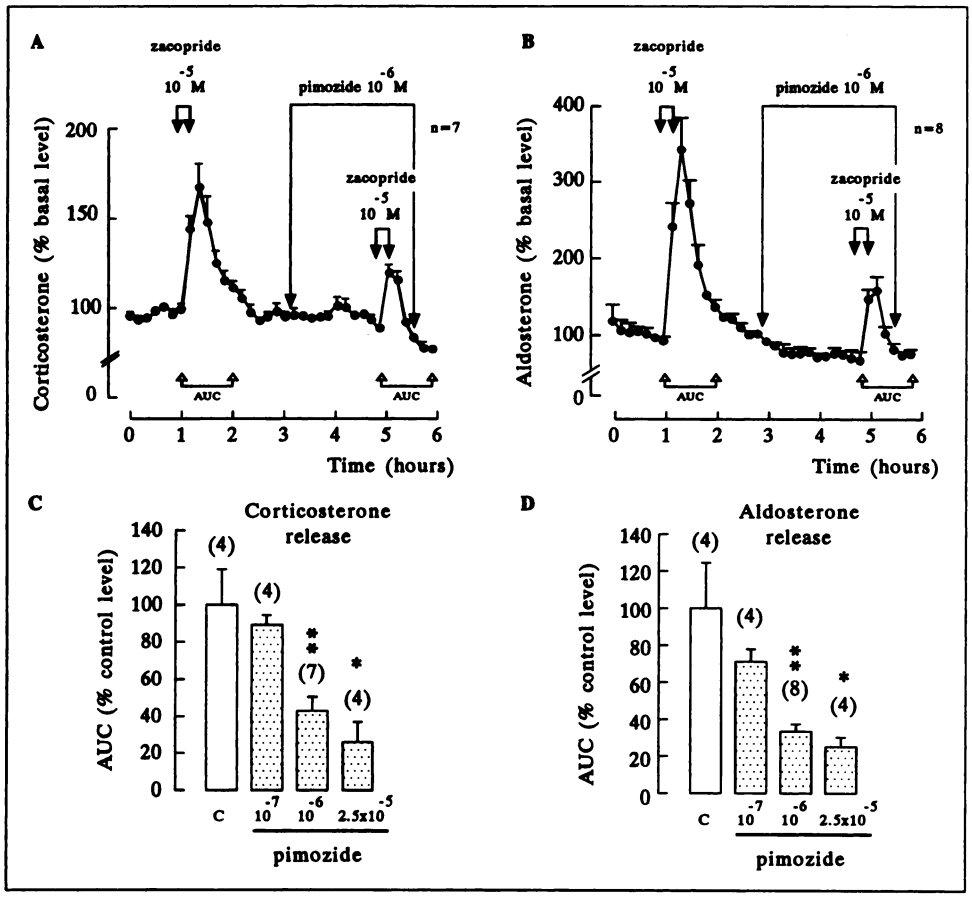

Fig. 15. Effect of the T-type calcium channel blocker pimozide on zacopride-induced steroid secretion by perifused adrenal slices. A and B, Effect of two pulses of zacopride (10^{-5} M; 20 min each) under normal conditions and during prolonged infusion of 10^{-6} M pimozide on corticosterone (A) and aldosterone (B) secretion. The mean secretion rates of corticosterone and aldosterone under basal conditions (100% basal level) were 22.7 ± 2.8 and 13.7 ± 2.3 pg/adrenal gland/min, respectively. (See legends to Fig. 11 for other designations.) C and D, Effects of increasing concentrations of pimozide on zacopride-induced stimulation of corticosterone (C) and aldosterone (D) secretion by perifused adrenal slices. Experimental values were calculated from data similar to those presented in A and B. Results are expressed as a percentage of the net increase of steroid secretion (AUC) induced by zacopride in the absence of pimozide. The net increases in corticosterone and aldosterone production (100% control AUC) were 3.5 ± 0.6 and 4.3 ± 0.7 ng/adrenal gland × min, respectively. *, $p < 0.05$; **, $p < 0.01$.

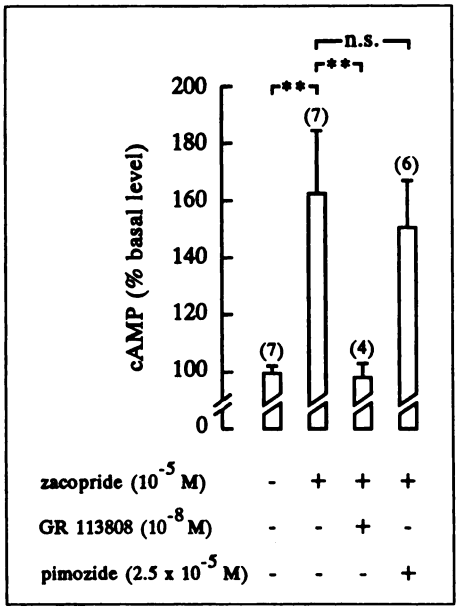

Fig. 16. Effect of zacopride (10^{-5} M) on cAMP production by frog adrenal slices in the presence of pimozide (2.5×10^{-5} M) or GR 113808 (10^{-8} M). Tissue cAMP content was measured after a 2-min incubation with the drug and calculated as a percentage of the control level. The number of independent experiments is indicated on each column. **, $p < 0.01$.

blocked the stimulatory action of 5-HT on $[Ca^{2+}]_i$ but did not affect the response of adrenocortical cells to the angiotensin II agonist [Sar^1,Val^5]AII. In agreement with this finding, we recently showed that the stimulatory effect of zacopride on corticosteroid secretion was inhibited in a dose-dependent manner by GR 113808 with a pK_i value of 10.3 (14).

Repeated pulses of 5-HT or zacopride resulted in a sequential increase in $[Ca^{2+}]_i$ with gradual attenuation of the response. This desensitization process is reminiscent of the tachyphylaxis of the secretory response observed during prolonged administration of 5-HT and/or zacopride to perifused adrenal slices (9, 13). Desensitization of the effects of 5-HT$_4$ receptor agonists has been reported in various systems, including mouse colliculi neurons (2), guinea pig ileum (29), and rat esophagus muscle (5). The mechanism responsible for homologous desensitization of 5-HT$_4$ receptors in colliculi neurons has been studied in detail by Ansanay *et al.* (30). These authors reported that the ability of 5-HT$_4$ receptor agonists to desensitize the adenylyl cyclase response is a function of their potency rather than of their efficacy. In agreement with this notion, the present study shows that 5-HT, which is more potent but less efficient than zacopride in stimulating corticosteroid secretion (14), also exhibits a higher desensitization index, as determined by the slope of the regression curves (−22.2 versus −13.1, respectively).

We next sought to determine the source of calcium involved in the elevation of $[Ca^{2+}]_i$ induced by 5-HT$_4$ receptor activation. Reduction of the calcium concentration in the bath solution from 1.3 mM to 8 nM by the addition of 10 mM EGTA totally suppressed the stimulatory effect of 5-HT on $[Ca^{2+}]_i$, suggesting that the increase in $[Ca^{2+}]_i$ can be ascribed only to calcium uptake. To further explore this hypothesis, we studied the effect of zacopride in the presence of Mn^{2+}, a calcium

substitute that avidly binds to indo-1 and quenches its fluorescence (31). The data revealed that exposure of the cells to zacopride with 6 mM Mn^{2+} in the bath solution produced a reduction in both the 405- and 480-mm fluorescence signals attributable to an influx of Mn^{2+} through calcium channels. In contrast, the effect of 5-HT was not blocked by thapsigargin, a calcium-ATPase inhibitor that causes depletion of intracellular calcium stores (32, 33) and totally blocks the calcium response of adrenal cells to ranakinin (25). In addition, the phospholipase C inhibitor U-73122 (34) did not affect the $[Ca^{2+}]_i$ rise evoked by zacopride, indicating that 5-HT$_4$ receptor agonists do not stimulate the inositol trisphosphate/Ca^{2+} transduction pathway. Altogether, these data indicate that in adrenocortical cells the increase in $[Ca^{2+}]_i$ associated with 5-HT$_4$ receptor activation can be accounted for exclusively by calcium influx through the plasma membrane. Previous studies have demonstrated that 5-HT, acting through 5-HT$_4$ receptors, stimulates calcium currents in human atrial myocytes (16). It has also been shown that 5-HT stimulates $^{45}Ca^{2+}$ uptake by rat glomerulosa cells, but this effect was blocked by the 5-HT$_{1/2}$ receptor antagonist methysergide (35). Thus, the present data provide the first evidence that activation of 5-HT$_4$ receptors in adrenocortical cells provokes an increase in calcium influx.

Our next objective was to investigate the possible involvement of calcium in the secretory response of adrenocortical cells to 5-HT$_4$ receptor activation. Suppression of calcium in the incubation medium caused a massive and transient increase in corticosteroid secretion from perifused adrenal slices, as previously described (36, 37). In calcium-free conditions, the stimulatory effect of zacopride on corticosterone and aldosterone secretion was significantly reduced. The attenuation of the response to the 5-HT$_4$ receptor agonist in the absence of calcium cannot be accounted for by exhaustion of the secretory activity of adrenocortical cells as administration of three consecutive pulses of 5-HT at 130-min intervals gives rise to a reproducible stimulation of corticosteroid secretion without tachyphylaxis (38). Similarly, the attenuation of the response to zacopride cannot be ascribed to the deleterious effect of calcium suppression because the stimulatory effect of CGRP (another corticotropic factor) is not impaired in calcium-free conditions.[1] In addition, administration of U-73122 (10^{-6} M) did not modify the secretory response of perifused adrenal slices to zacopride, whereas at the same concentration, U-73122 suppressed the stimulatory effect of tachykinins on frog adrenal cells (25). The requirement of calcium for the 5-HT-induced aldosterone secretion from rat glomerulosa cells has been reported (39). These data demonstrate that the $[Ca^{2+}]_i$ rise induced by 5-HT$_4$ receptors mediates, at least in part, the stimulatory effect of 5-HT on corticosteroid secretion.

To determine which type of calcium channel was responsible for the calcium entry evoked by 5-HT$_4$ receptor agonists, we studied the effect of voltage-operated calcium channel blockers on $[Ca^{2+}]_i$ rise and corticosteroid secretion induced by zacopride. Preincubation of cultured adrenocortical cells with ω-conotoxin GVIA significantly reduced the basal $[Ca^{2+}]_i$, whereas nifedipine and pimozide had no effect on basal $[Ca^{2+}]_i$ in adrenal cells, indicating that N-type calcium channels selectively contribute to the maintenance of calcium homeostasis in resting conditions. Consistent with the secretion-coupling concept (40), ω-conotoxin also caused a marked and sustained decrease in the spontaneous rate of corticosteroid secretion. In the presence of nifedipine or ω-conotoxin, the $[Ca^{2+}]_i$ rise induced by zacopride was not altered. In contrast, pimozide, which is considered to be a selective blocker of T-type calcium channels (23, 41), abolished the $[Ca^{2+}]_i$ response induced by zacopride and inhibited, in a dose-dependent manner, the stimulatory effect of zacopride on corticosteroid secretion. However, pimozide possesses a broad pharmacological spectrum. In particular, pimozide acts as a 5-HT$_{2A}$ receptor antagonist (42). It was thus essential to verify that pimozide, at the concentration used, did not directly interact with the 5-HT$_4$ receptor. The data reported in Fig. 16 show that pimozide did not inhibit the stimulatory effect of zacopride on cAMP production. In addition, pimozide did not affect the binding of [^{3}H]GR 113808 on membranes from mouse embryo colliculi (data not shown). It appears, therefore, that the blockage of the $[Ca^{2+}]_i$ and secretory responses induced by pimozide could not be accounted for by a nonspecific interaction with 5-HT$_4$ receptors. The notion that a T-type calcium channel is involved in the mechanism of action of 5-HT in frog adrenocortical cells is consonant with a recent report that showed that T-type calcium channels mediate the stimulatory effect of adrenocorticotropic hormone on cortisol secretion in bovine adrenocortical cells (43). In contrast, electrophysiological studies have shown that in human atrial myocytes, 5-HT$_4$ receptor agonists stimulate an L-type calcium current (16). Taken together, these data re-

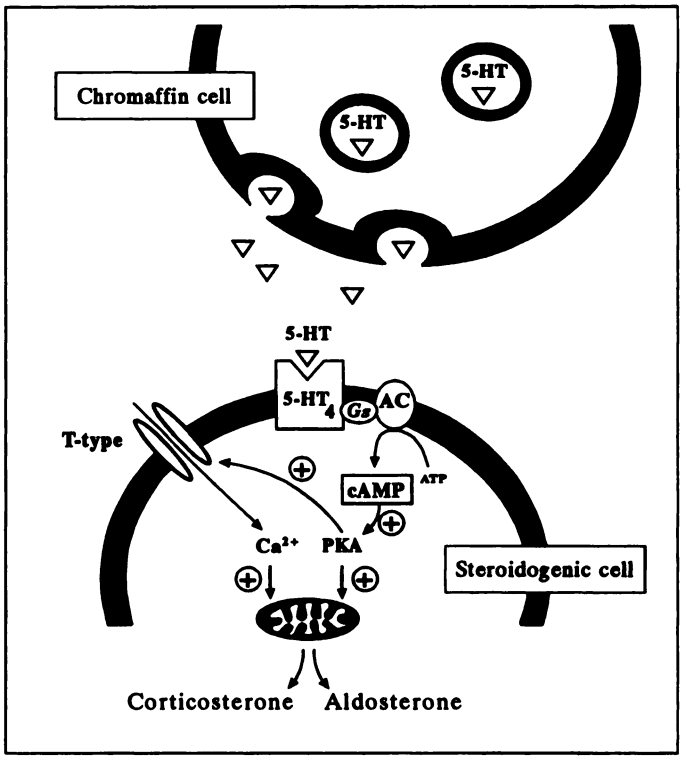

Fig. 17. Schematic summarizing the mechanism of action of 5-HT in the frog adrenal gland. Chromaffin cells synthesize and release 5-HT in the vicinity of steroidogenic cells (46). Activation of the 5-HT$_4$ receptor increases adenylyl cyclase activity, which in turn stimulates calcium influx through T-type calcium channels. Both the increase in cAMP formation and the $[Ca^{2+}]_i$ rise contribute to stimulation of corticosterone and aldosterone secretion.

[1] M. Esneu, C. Delarue, H. Vaudry, unpublished observations.

veal that stimulation of 5-HT_4 receptors in different cell types is associated with activation of distinct types of voltage-operated calcium currents.

In adrenocortical cells, 5-HT_4 receptor agonists cause both an increase in cAMP formation (9, 13) and a rise in $[Ca^{2+}]_i$ (present study). The final goal of our study was thus to determine the sequence of events associated with activation of 5-HT_4 receptors. In human atrial myocytes (16) and rat hippocampal neurons (44), the stimulatory effect of 5-HT_4 receptor agonists on calcium influx is mediated through activation of the adenylyl cyclase/PKA pathway. Several lines of evidence indicate that in adrenal cells, the increase in $[Ca^{2+}]_i$ is also mediated by a cAMP-dependent protein kinase. (i) In contrast to most corticotropic factors, which cause an immediate increase in $[Ca^{2+}]_i$ in frog adrenocortical cells (18, 45), the calcium response to 5-HT was generally delayed by 30–60 sec, suggesting that it could be secondary to activation of adenylyl cyclase. (ii) Administration of dbcAMP, a permeant analogue of cAMP, mimicked the stimulatory effect of 5-HT and zacopride on $[Ca^{2+}]_i$. (iii) The lag period was shorter after the application of dbcAMP than after the application of 5-HT_4 receptor agonists. (iv) The effects of zacopride and dbcAMP on $[Ca^{2+}]_i$ rise were not additive, suggesting that both compounds act through the same mechanism. (v) The stimulatory effect of zacopride on $[Ca^{2+}]_i$ was potentiated by the phosphodiesterase inhibitor IBMX. (vi) In contrast, the zacopride-induced stimulation of $[Ca^{2+}]_i$ was markedly attenuated in the presence of the PKA inhibitor Rp-cAMPS. Taken together, these observations indicate that the $[Ca^{2+}]_i$ rise is secondary to the activation of the cAMP/PKA pathway.

A proposed model illustrating the mechanism of action of 5-HT on adrenocortical cells is shown in Fig. 17. It has previously been shown that frog chromaffin cells contain (46), synthesize, and release 5-HT (47) in the vicinity of adrenocortical cells. 5-HT stimulates 5-HT_4 receptors positively coupled to adenylyl cyclase (9, 13). Subsequently, a cAMP-dependent protein kinase is responsible for the activation of T-type calcium channels. Both the increased formation of cAMP and the resulting calcium influx appear to be involved in the stimulatory effect of 5-HT on corticosteroid secretion.

Acknowledgments

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