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Calcium Ionophore A23187 Induces Expression of Glucose-Regulated Genes and Their Heterologous Fusion Genes

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Using two cDNA clones which encode hamster genes specifically induced by glucose starvation, we demonstrated that an 8- and 30-fold increase, respectively, in the transcription rates of these genes was coordinately effected by calcium ionophore A23187 treatment, resulting in a similar increase in the steady-state levels of their mRNAs. This response was observed within several hours of ionophore treatment in several mammalian cell types and appeared to be specifically mediated by A23187 but not by other ionophores in general. To define the regulatory sequence which mediates this Ca^{2+}-induced response, we showed by gene transfection techniques that the 5′ flanking sequence of a rat glucose-regulated gene contained the region for induction by A23187. The system reported here offers attractive features for the study of specific gene regulation by Ca^{2+}.

Compared with the enormous amount of information available on the regulation of cellular activities by calcium (4), very little is known about the role of calcium in the control of gene expression. Since abrupt changes in the cytoplasmic Ca^{2+} concentration have been shown to affect growth rate, intermediary metabolism, electrical activities, and cell movements, specific mechanisms must exist to regulate the physiological Ca^{2+} concentration inside the cell. To understand the molecular basis of the regulatory role of intracellular Ca^{2+}, a first step is to identify cellular proteins whose synthesis is regulated by changes in the Ca^{2+} concentration. We and others have examined the pattern of protein synthesis in a variety of cells to investigate the influence of the extracellular Ca^{2+} concentration and of Ca^{2+} ionophores on the synthesis of specific proteins. Two proteins have recently been identified whose synthesis is specifically stimulated by the calcium ionophores ionomycin and A23187 (10, 14, 21, 24). The same proteins can also be induced by shifting cells from low Ca^{2+} (0.15 mM) to normal Ca^{2+} (1.8 mM) medium (14). These are the 94- to 100-kilodalton and 78- to 80-kilodalton proteins whose synthesis is stimulated by the absence of glucose in the culture medium; they are generally referred to as the glucose-regulated proteins (GRPs) (15, 16, 18).

Using the hamster mutant cell line K12, which overproduces these GRPs at the nonpermissive temperature, we previously isolated two cDNA clones, p4A3 and p3C5, which by the criterion of hybrid select translation encode the hamster GRPs of 94 and 78 kilodaltons (GRP94 and GRP78), respectively (11, 12). These cDNAs are encoded by single-copy genes whose transcription is highly inducible by glucose starvation in a variety of animal cells (13). In this report, we demonstrate that within a few hours, an 8- and 30-fold increase in the transcription rates of these genes can be coordinately effected by calcium ionophore A23187 treatment, resulting in a similar increase in the steady-state levels of their mRNAs. This response can be observed in a variety of cell types derived from different species.

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Further characterization of the promoter-regulatory sequences which may mediate these responses requires isolation of the p3C5 and p4A3 structural genes and their flanking sequences. Therefore, we have isolated the rat structural gene encoding the analogous GRP78 and constructed a hybrid gene (pNE/12) containing the 5′ sequence of the rat gene fused to the bacterial neomycin resistance (*neo*) gene (3). After transfection into hamster fibroblasts, the *neo* transcripts were induced to high levels by glucose starvation; the hybrid gene could also be regulated by temperature when it was introduced into the temperature-sensitive mutant cell line K12 (3). Here we show that the rat-*neo* hybrid mRNA can also be regulated by A23187. Further, we have constructed a new hybrid gene (pI10) by fusing a 1.25-kilobase (kb) fragment of the 5′ flanking sequence of the rat GRP78 gene to the bacterial chloramphenicol acetyltransferase (CAT) gene (6). In a transient assay, we detected a 20-fold increase in CAT activity when the transfected cells were treated with A23187. Therefore, the regulatory element required for A23187 induction is contained within the 5′ flanking sequence of the rat GRP78 gene.

MATERIALS AND METHODS

Cell lines, media, and culture conditions. The Chinese hamster fibroblast cell lines Wg1A and K12 have been described previously (9, 17). K12 is a temperature-sensitive mutant derived from the parental cell line Wg1A. The phenotypes of K12 and Wg1A are similar when the cells are incubated at the permissive temperature, 35°C. The cells are routinely maintained in Dulbecco modified Eagle medium (DMEM) containing 4.5 mg of glucose per ml and supplemented with 10% cadet calf serum.

The normal rat kidney (NRK) cell line was obtained from P. Vogt and was maintained in DMEM supplemented with 10% cadet calf serum.

The human embryo kidney cells (cell line 293) were obtained from M. Karin and maintained in DMEM supplemented with 10% fetal calf serum.

The mouse fibroblast cell line (cell line LA9) was obtained

from R. E. K. Fournier and was a hypoxanthine phosphoribosyltransferase-negative derivative of mouse L cells. The cells were maintained in DMEM-F12 (1:1) medium supplemented with 10% fetal calf serum.

Plasmid DNA. The construction of the hamster cDNA plasmids p3C5 and p4A3, encoding hamster GRP78 and GRP94, respectively, has been described previously (11, 12).

The pSVOCAT plasmid was provided by B. Howard. The plasmid contains the bacterial CAT gene inserted into pBR322. A *Hin*dIII site is located 5′ to the CAT gene, where heterologous promoter sequences can be inserted. Construction of this plasmid has been described (6).

Conditions for ionophore treatment. Cells were seeded in 150-mm-diameter dishes at a density of 1.5×10^4 cells per cm^2. When the cells reached 80 to 90% confluency, fresh medium was added to ensure that the cells were not starved of glucose at the time of ionophore treatment. The final concentrations used for the ionophores were: A23187, 7 μM; valinomycin, 2 μM; gramicidin, 2 μM; and nigericin, 1 μM. At various times after addition of the ionophores, total cytoplasmic RNA was extracted as previously described (11).

RNA gel blots. Conditions for the isolation of cytoplasmic RNA, electrophoresis on formaldehyde-formamide gel, and transfer to nitrocellulose filters have been described (11). For hybridization to the cDNA plasmids p3C5, p4A3, and p3A10, plasmid DNA was labeled by nick-translation to specific activities of 2×10^7 to 5×10^7 cpm/μg of DNA. Hybridization and washing conditions were as described (11).

In vitro transcription measurement. The procedure for in vitro labeling of nuclear RNA was a modification of a previously described method (2). Wg1A cells were grown to 80 to 90% confluency in 150-mm-diameter culture dishes. After a change to fresh medium, A23187 was added to a final concentration of 7 μM and incubated for various times (two dishes per time point). Incubations were terminated by suspending the cells in reticulocyte standard (RS) buffer (10 mM Tris-hydrochloride [pH 7.4], 10 mM NaCl, 3 mM $MgCl_2$) containing 0.1 mM $CaCl_2$, and the cells were centrifuged. To isolate nuclei, the pellet was suspended twice in RS buffer containing 0.1 mM $CaCl_2$ and 0.5% (vol/vol) Nonidet P-40 and centrifuged. The nuclei pellet was then resuspended in RS buffer containing 0.1 mM $CaCl_2$ but without Nonidet P-40 and centrifuged. The nuclei (200 μg of DNA in a volume of 50 μl) were suspended in 50 μl of nuclei storage buffer (40% glycerol, 50 mM Tris-hydrochloride [pH 8.3], 5 mM $MgCl_2$, 0.1 mM EDTA). A reaction mixture (100 μl) was added to the nuclei suspension, having a final concentration of 25% glycerol, 2 mM $MgCl_2$, 2.5 mM dithiothreitol, 70 mM KCl, 0.25 mM GTP, 0.25 mM CTP, 0.5 mM ATP, and 250 μCi of [α-^{32}P]UTP (3,000 Ci/mmol). This transcription reaction mixture was incubated at 37°C for 15 min, and the reaction was terminated by adding 100 volumes of cold RS buffer containing 0.1 mM $CaCl_2$. Nuclear RNA was isolated as described previously (13). The nuclei were suspended in 3 ml of high-salt buffer (0.5 M NaCl, 10 mM Tris-hydrochloride [pH 7.5], 50 mM $MgCl_2$, 2 mM $CaCl_2$) containing 60 μg of pancreatic DNase. After incubation at 65°C for 15 s with agitation, the reaction was terminated by the addition of 60 μl of 20% sodium dodecyl sulfate, 60 μl of 0.5 M EDTA, 66 μl of 3 M sodium acetate (pH 5.4), and 5 ml of 60 mM sodium acetate (pH 5.4)–10 mM EDTA. Yeast tRNA (400 μg) was added as the carrier, and the solution was extracted twice with phenol-chloroform-isoamyl alcohol (25:24:1) preequilibrated with 60 mM sodium acetate (pH 5.4)–10 mM EDTA and once with chloroform. RNA was precipitated by adding 0.1 volume of 3 M sodium acetate (pH 5.4) and 2 volumes of 95% ethanol at −20°C overnight. The RNA precipitate was pelleted and suspended in 0.5 ml of 20 mM TES buffer [2-(2-hydroxy-1,1-bis(hydroxymethyl)ethylamino) ethanesulfonic acid, pH 7.4].

The conditions for the hybridization of labeled nuclear RNA to nitrocellulose filters containing excess DNA were previously described (11). Quarter pieces of the filters used contained 25 μg of plasmid DNA. Plasmid pBR322 was used for background binding. Blank filters which contained no DNA were also used to monitor nonspecific binding. Hybridization reaction mixtures contained either 0.9×10^6 or 1.8×10^6 cpm of labeled nuclear RNA.

Construction of hybrid genes. The construction of pNE/12 has been described (3). Essentially, a 6.2-kb *Bam*HI fragment containing the 5′ sequence of the rat GRP78 gene was fused 5′ to the bacterial neomycin resistance gene (*neo*) contained within plasmid pNEO3.

To construct pI10, pUC 6.2, a pUC8 plasmid containing the 6.2-kb *Bam*HI fragment from the rat GRP78 gene subcloned into the *Bam*HI site of its polylinker sequence (3), was opened at the single *Sph*I site. This *Sph*I site was located about 1.45 kb 5′ to the TATA sequence (A. Lin and A. S. Lee, manuscript in preparation; see Fig. 5). After Bal 31 treatment (2 min at 37°C, 1.6 U of Bal 31 nuclease per 12 μg of DNA), the DNA was treated with *Sal*I, and the 5′ protruding ends were filled by the Klenow fragment of DNA polymerase I. The large fragment was recircularized by bacteriophage T4 ligase and used for transformation into *Escherichia coli* HB101 cells by the Kushner procedure (8). Under this Bal 31 digestion condition, about 0.2 kb was digested from either side of the *Sph*I site. Thus, one of the transformants isolated, designated p2I, after digestion with *Bss*hII and *Pst*I generated three fragments of 3.4, 2.8, and 1.25 kb. The 1.25-kb *Pst*I-*Bss*hII fragment contained the 5′ flanking sequence of the rat GRP78, including the TATA sequence, which was located about 20 nucleotides 5′ to the *Bss*hII site. This fragment was recovered from a 1% low-melting-point agarose gel and treated with the Klenow fragment of DNA polymerase I before ligation to the pSVOCAT plasmid linearized at the unique *Hin*dIII site. To eliminate vector self-ligation, pSVOCAT linear DNA was treated with bacterial alkaline phosphatase and the 5′ ends were filled by the Klenow fragment of DNA polymerase I. The resulting recombinants were transfected into *E. coli* HB101 cells. The transformants were screened with a 1.1-kb *Pvu*II subfragment of pUC6.2 which contained the 5′ sequence of the rat GRP78 gene (3). One of the transformants, designated pI10, contained the 1.25-kb *Pst*I-*Bss*hII fragment fused in the same orientation of transcription as the CAT gene. The orientation of transcription was determined from *Stu*I and *Nde*I digestions (see Fig. 5).

Transformation conditions. The conditions for DNA transfection have been described (3, 20). Essentially, the transfecting DNA was mixed with 7 μg of high-molecular-weight HeLa cell DNA as carrier in the transformation buffer and added to either K12 or Wg1A cells grown as monolayers. Incubation was at 35°C for 20 min. At that time, 10 ml of fresh DMEM was added to the cells, and incubation was continued for 4 or 16 h at 35°C before glycerol shock. For the selection of stable transformants, G418 was added to 400 μg/ml 2 days after transfection. Individual G418-resistant colonies were picked after 3 to 4 weeks and expanded into mass culture. For transient transformants, the cells were harvested 44 h after transfection without any selection.

Assay for CAT activity. The level of CAT activity was determined by the procedure of Gorman et al. (6) with the following modifications. After transfection, the cells from each 100-mm-diameter dish were washed three times with phosphate-buffered saline and suspended in 50 μl of 0.25 M Tris-hydrochloride buffer, pH 7.8. The cells were disrupted by three freeze-thaw cycles. The cell debris was spun down in a microfuge, and the supernatant was assayed for the amount of protein by the Bio-Rad protein assay. For assaying the CAT activity, the final volume was 75 μl, which contained 0.25 μCi of [^{14}C]chloramphenicol (57.8 mCi/mmol; New England Nuclear Corp.), and the amount of extract added varied from 2 to 7 μl. The reaction was stopped at the end of 30 min at 37°C. The samples were extracted with ethyl acetate, dried, and spotted on silica gel thin-layer chromatography plates. After the plates were developed in chloroform-methanol (95:5), the thin-layer chromatography plate was exposed to XAR-2 Kodak film for 24 h. For quantitation of the acetylation reaction, spots corresponding to the unacetylated and acetylated forms were cut out, and their radioactivity was counted in a liquid scintillation counter. The percentage of conversion to the acetylated form was calculated by dividing the counts per minute in the two acetylated forms by the total counts per minute (both acetylated and nonacetylated forms). The fold induction of CAT activity was calculated as the ratio of conversion between the induced and the noninduced conditions.

RESULTS

Kinetics of induction of p3C5 and p4A3 mRNA by A23187. To determine whether the increased amount of GRP94 and GRP78 caused by A23187 treatment was due to transcriptional or posttranscriptional control, we measured the kinetics of their mRNA accumulation after A23187 treatment in a Chinese hamster fibroblast cell line, Wg1A. Equal amounts of cytoplasmic RNA isolated from cells treated with 7 μM A23187 for various intervals were size-separated on denaturing gel, transferred to nitrocellulose paper, and hybridized to radiolabeled cDNA plasmids p3C5 and p4A3. Another cDNA clone, p3A10, randomly chosen from the same cDNA library, whose expression was not affected by glucose, was added as an internal control. There was a steady increase in the level of p3C5 and p4A3 transcripts within 3 h of treatment of the hamster fibroblasts with A23187 (Fig. 1). By 6 h, a 32-fold increase in the p3C5 mRNA level and a 10-fold increase in the p4A3 mRNA level was observed. In contrast, the level of p3A10 remained constant throughout ionophore treatment. Compared with the results of our previous studies, the magnitude of the response to A23187 was three times greater and the onset of response occurred 10 h earlier than that induced by glucose starvation (13). Based on our measurements of the steady-state levels of p3C5 and p4A3 mRNAs (11), we estimated that at the plateau levels, the mRNA of p3C5 and p4A3 induced by A23187 constituted about 3.5 and 1%, respectively, of the total polyadenylated RNA of the cells.

Rate of transcription of p3C5 and p4A3 mRNA. To determine whether the increase in the p3C5 and p4A3 mRNA levels by treatment with the ionophore was due to new synthesis, we measured the transcription rates of these genes in the presence of 7 μM A23187, which we determined to be the most effective concentration of this ionophore (data not shown). At various intervals, nuclei were isolated from cells treated with A23187, and in vitro-labeled heterogeneous nuclear RNA was hybridized with p3C5 and p4A3 DNA bound to nitrocellulose filters. Filters containing pBR322 DNA were added as the control (Table 1). After a lag of about 2 h, we detected a 30-fold increase in the transcription rate of p3C5 and an 8-fold increase in that of p4A3. Since a similar increase was observed in their mRNA levels, these genes are regulated largely at the transcriptional level.

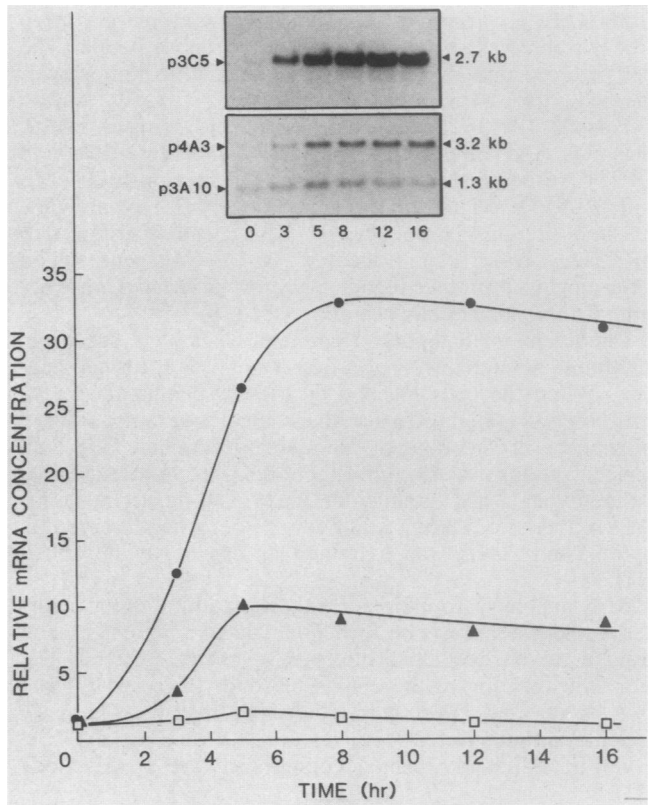

FIG. 1. Kinetics of accumulation of p3C5 and p4A3 mRNA. At the time points indicated, cytoplasmic RNA was extracted from Wg1A cells treated with 7 μM A23187. RNA (10 μg) from each time point was applied to a denaturing formaldehyde-formamide RNA gel, electrophoresed, and blotted onto nitrocellulose filters. The filters were hybridized with nick-translated p3C5 (●), p4A3 (▲), and p3A10 (□) DNA. The autoradiograms (inset) were quantitated by densitometry to obtain the relative levels of p3C5, p4A3, and p3A10 mRNAs at different times of incubation with A23187.

Specificity of induction by A23187. The specificity of induction by A23187 was examined by comparing the mRNA levels of p3C5 and p4A3 in cells treated with other ionophores, such as the potassium ionophores valinomycin and nigericin, and the more general monovalent cation ionophore gramicidin (19). Nigericin is known to perturb the pH gradient in membranes, whereas valinomycin can cause changes in membrane potential. The p3C5 and p4A3 mRNA levels were not significantly affected by any of these ionophores (Fig. 2), suggesting that general effects caused by ionophore treatment, such as K^+-H^+ exchange, electrical potential, and shift in pH, were not involved. Our results confirm the previous observations that the effect of Ca^{2+} ionophores on GRP94 and GRP78 protein synthesis is not due to changes in the Na^+ concentration of the cells or as a result of uncoupling of oxidative phosphorylation (14).

General occurrence of the response. To test whether the effect of A23187 on the accumulation of the p3C5 and p4A3

TABLE 1. In vitro transcription rates of p3C5 and p4A3 during treatment with A23187

Time[a]	Input (10^6 cpm)	cpm bound[b]					Relative transcriptional rate[c]	
		pBR322	p3C5	Weighted avg	p4A3	Weighted avg	p3C5	p4A3
0	0.9	—[d]	24	20	4	3.7	1	1
	1.8	2	30		6			
0.5	0.9	0	32	19.2	4	3.3	1	1
	1.8	4	20		5			
2	0.9	–	506	558	18	24.8	27.5	6.7
	1.8	12	1,001		49			
4	0.9	–	542	601	20	30	30	8.1
	1.8	21	1,082		60			
7	0.9	–	593	603	25	31.8	30	8.6
	1.8	13	1,036		61			

[a] Wg1A cells were grown to 80% confluency in complete DMEM. At time zero, A23187 was added to all cells. At various times after the addition, the nuclei were isolated and the nuclear RNA was labeled in vitro.

[b] Calculated as counts subtracted from those of the blank filters, which was about 50 cpm. Weighted average, counts per minute bound per 10^6 input cpm, calculated by $(A+B)/C$, where A is counts per minute bound per filter with 0.9×10^6 input cpm, B is counts per minute bound per filter with 1.8×10^6 input cpm, and C is total input counts per minutes (2.7×10^6 cpm).

[c] The relative transcriptional rate is calculated by dividing the weighted average value at each time point by the weighted average value at time zero.

[d] —, Not done.

transcripts is tissue specific or species specific, we treated cultures of rat kidney fibroblasts (NRK), mouse connective tissue fibroblasts (LA9), human embryo kidney cells (293), and Chinese hamster lung fibroblasts (Wg1A) with A23187. Cytoplasmic RNA was extracted from these cells, and the levels of p3C5 and p4A3 mRNA were monitored by RNA blot hybridization (Fig. 3). In each of the cell lines tested, there was a 10- to 30-fold increase in p3C5 and p4A3 mRNA levels when the cells were treated with A23187. Therefore, this Ca^{2+} ionophore-induced response occurs in several cell types derived from different mammalian species. The facts that the p3C5 and p4A3 mRNAs produced by the various animal species are very similar, if not identical, in size and that they cross-hybridized efficiently with the hamster cDNA probes strongly suggest that these mRNA sequences are conserved.

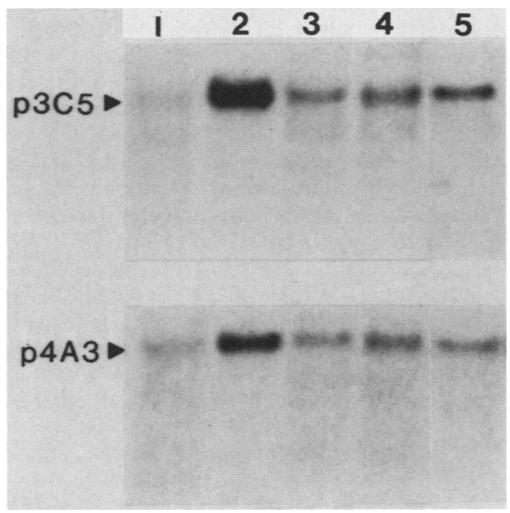

FIG. 2. Effect of various ionophores. K12 (lanes 1 to 4) and Wg1A (lane 5) cells were incubated in complete DMEM (lane 1) or in DMEM supplemented with 7 μM A23187 (lane 2), 2 μM valinomycin (lane 3), 2 μM gramicidin (lane 4), or 1 μM nigericin (lane 5). Incubation was for 16 h at 35°C. After the various treatments, total cytoplasmic RNA was extracted from the cells and subjected to RNA blot analysis as described in the legend to Fig. 1. The autoradiograms are shown.

Regulation of a heterologous gene by A23187 in stable transformants. We previously described the construction of a hybrid gene (pNE/12) which contained about 3 kb of the 5′ flanking sequence and 3 kb of the 5′ sequence of the rat GRP78 gene fused to the bacterial neomycin resistance gene (*neo*) (3). The structure of pNE/12 is shown in Fig. 4A. To test whether the *neo* mRNA could also be regulated by Ca^{2+} in this hybrid gene, we isolated total cytoplasmic RNA from stable transformants treated with A23187 and, for comparison, from the same cells treated with dibutyryl cyclic AMP (cAMP), gramicidin, or glucose-free medium. The endogenous mRNA levels of p3C5, p3A10, and the rat-*neo* fusion

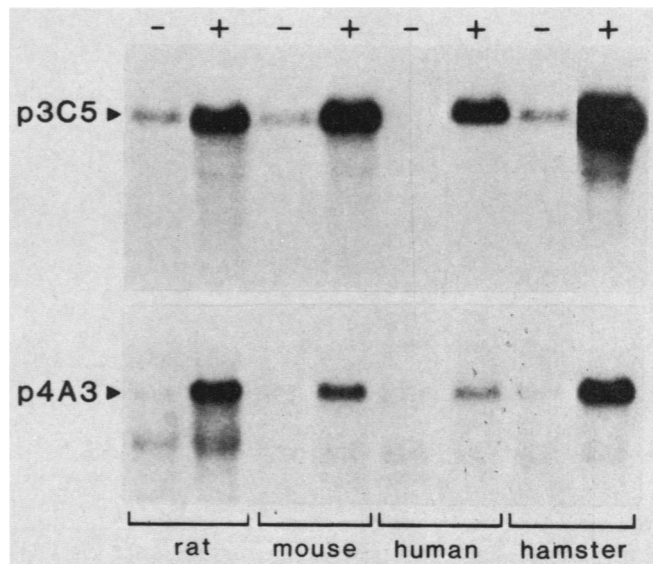

FIG. 3. Effects of A23187 on cells from different species and tissues. Cell lines derived from rat kidney, mouse fibroblast, human embryo kidney, and hamster lung cells were incubated in DMEM in the absence (−) or presence (+) of 7 μM A23187 for 8 h. At the end of the incubation period, cytoplasmic RNA was extracted and subjected to RNA blot analysis as described in the legend to Fig. 1. RNA blots were hybridized with nick-translated p3C5 and p4A3 DNA. The autoradiograms are shown.

gene were monitored. An example of the RNA blot analysis is shown in Fig. 4B. As expected, the endogenous level of p3C5 mRNA was elevated 30-fold by A23187 treatment and 10-fold by glucose starvation, but was relatively unaffected by dibutyryl cAMP or gramicidin. The levels of p3A10 remained the same in all samples. Similar to the endogenous p3C5 gene, the rat-*neo* fusion mRNA level was increased 25-fold by A23187 treatment and 5-fold by glucose starvation and was unaffected by dibutyryl cAMP and gramicidin.

Regulatory sequence contained within the 5′ flanking sequence. To test whether the regulatory sequences for the Ca^{2+}-induced response reside at the 5′ flanking sequence of the gene, we used a combination of restriction digests to subclone a 1.25-kb fragment containing the 5′ flanking and TATA sequences of the rat GRP78 gene into the unique *Hin*dIII site of pSVOCAT. The construction scheme for the resulting plasmid, pI10, is shown in Fig. 5. This plasmid was used to transfect K12 cells, and after 28 h fresh DMEM or fresh DMEM containing A23187 was added to identical sets of transfected K12 cells. Cell extracts were prepared after 16 h of treatment at 35°C and assayed for CAT activity (Fig. 6). It is evident that the 1.25-kb 5′ flanking sequence contains a functional regulatory-promoter sequence for the Ca^{2+}-induced response. At 35°C in regular medium, a basal activity was detected. After treatment with 7 μM A23187 for 16 h, a 20-fold increase in CAT activity was detected. Thus, the A23187 effect was observed both in stable transformants in which the fusion genes have been integrated into the host chromosomes and in transient transformants in which the fusion genes probably exist extrachromosomally.

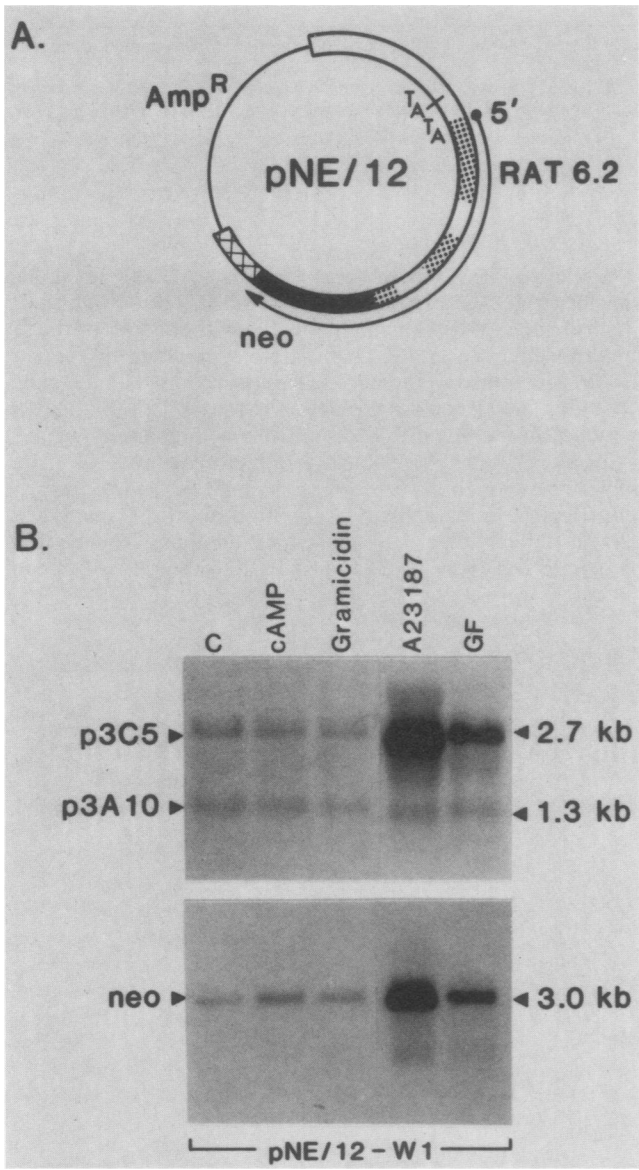

FIG. 4. Regulation of *neo* mRNA levels in the hybrid gene. (A) Structure of the rat-*neo* hybrid gene pNE/12. The construction of this hybrid gene has been described (3). pNE/12 contains about 3 kb of the 5′ flanking sequence and 3 kb of the 5′ sequence of the rat gene encoding GRP78 fused to the neomycin resistance gene (*neo*). The position of the TATA box and the direction of transcription are shown. Dotted area, Region of homology with p3C5; solid black area, *neo*-coding sequences; crosshatched area, polyadenylation site; line, pBR322 sequence. (B) Levels of p3C5, p4A3, and rat-*neo* hybrid mRNAs in a Wg1A stable transformant pNE/12-W1. The cells were incubated in complete DMEM (lane C), in DMEM supplemented with 0.1 mM dibutyryl cAMP (cAMP), in DMEM supplemented with 1 μM gramicidin, in DMEM supplemented with 7 μM A23187, or in glucose-free DMEM (GF). Treatment was for 16 h at 35°C. Conditions for the preparation of the GF medium were as described (13). The probe for *neo*-specific mRNA was prepared from *Pvu*II fragments derived from pNEO3 as described (3). The autoradiograms are shown.

DISCUSSION

The influence of extracellular Ca^{2+} on the secretion and production of prolactin and parathyroid hormones in specialized tissues such as the pituitary and parathyroid glands is a well-known phenomenon (7, 23). Although it has been shown that Ca^{2+} interacts synergistically with growth factors and hormones to increase transcription of the prolactin gene (22), the molecular mechanism of the Ca^{2+} involvement in these prolonged induction responses remains unknown. Compared with these systems, the observed stimulation of the synthesis of GRP94 and GRP78 occurs generally in common cell types and appears to be a direct response of the cells to a sudden change in the intracellular calcium concentration. Our results extend those of the previous studies to the transcriptional level and demonstrate for the first time that an 8- and 30-fold increase in the transcription of two specific cellular genes could be coordinately effected by A23187 treatment within a 3-h period. The identification of a regulatory-promoter sequence responding to A23187 treatment or a change in the Ca^{2+} concentration would be a first step in defining the components necessary for this response. Deletion analysis of the rat gene sequence would enable us to define the exact region required for the induction, and DNA-protein binding studies may yield information on the protein factor(s) involved.

Although the cellular function served by GRP94 and GRP78 is unknown, a partial amino-terminal sequence for both proteins isolated from hamster cells has been determined (11). Interestingly, both GRP94 and GRP78 contain amino-terminal sequences which are unusually rich in aspartic acid and glutamic acid residues. Several Ca^{2+}-binding proteins, such as calmodulin and troponin C, are also rich in glutamic and aspartic acids (5). Thus, one could speculate that the acidic residues of GRP94 and GRP78 at the amino terminus may well form a highly negatively charged environ-

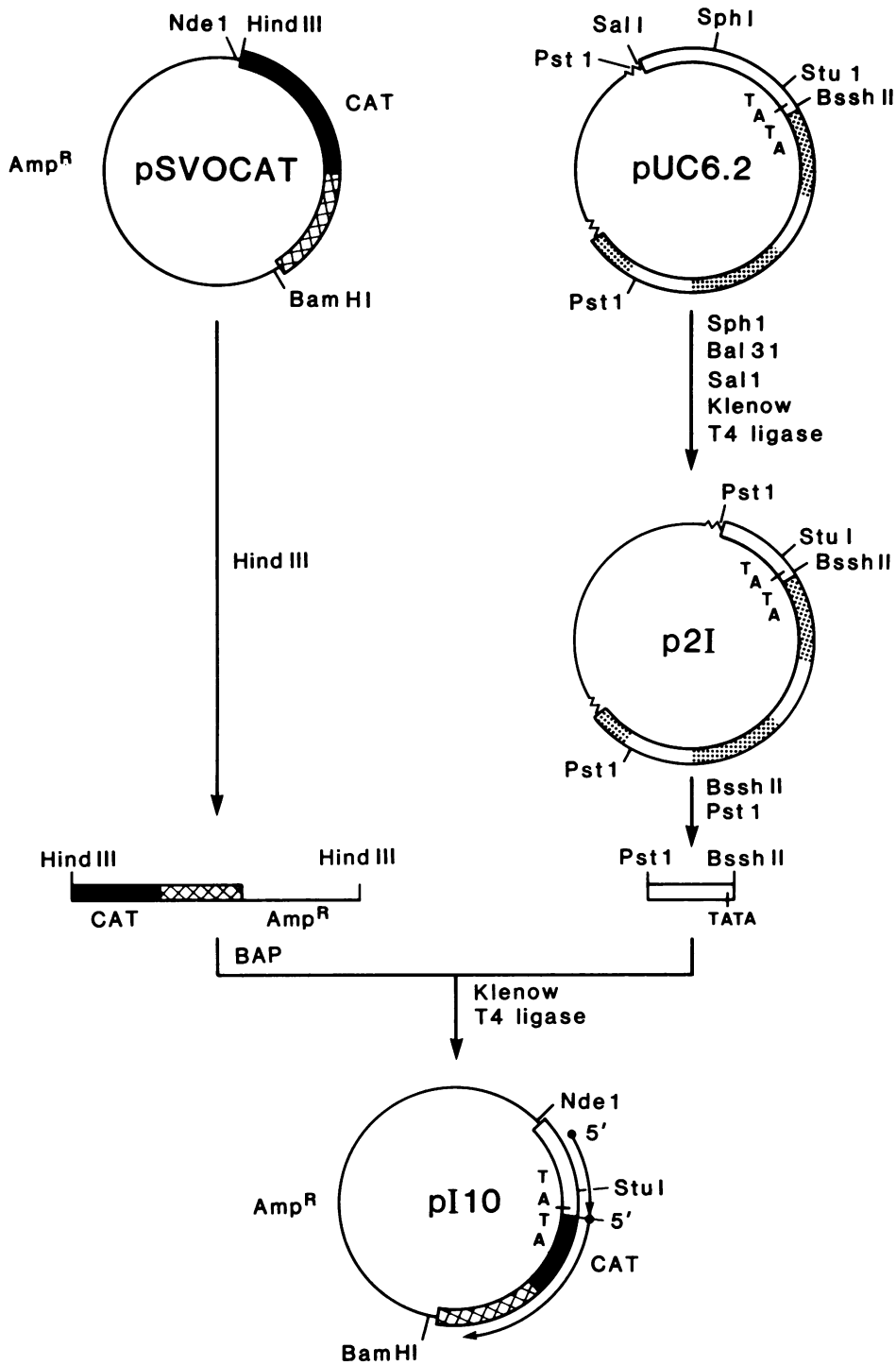

FIG. 5. Construction of pI10. The 5′ flanking sequence is shown as an open box. The location of the TATA sequence is indicated. Dotted area, Sequence of homology to p3C5 sequences; solid black area, bacterial CAT gene; crosshatched area, simian virus 40 polyadenylation site; wavy line, polylinker sequences in pUC6.2 derived from pUC8. The orientation of transcription for pI10 is indicated. BAP, Bacterial alkaline phosphatase.

ment that will effectively bind Ca^{2+}, which in turn autoregulates the synthesis of their mRNA through a protein intermediate. Further investigation into the physiological roles played by these proteins will help test this hypothesis.

As we have shown earlier that both GRP94 and GRP78 can be regulated by glucose starvation (10, 13), an interesting question arises: what is the relationship between glucose starvation and A23187 treatment that results in the induction of the same genes? Recently, it has been demonstrated that

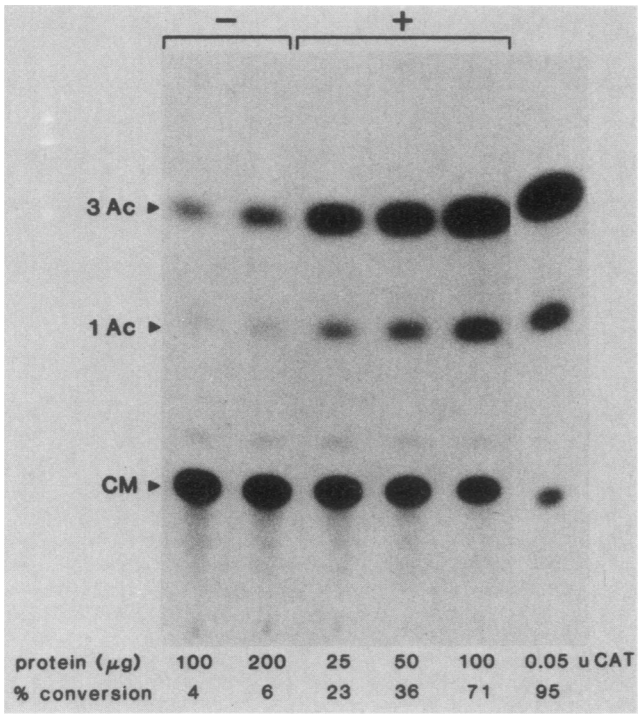

FIG. 6. Inducibility of pI10 by calcium ionophore A23187. pI10 (3 μg) was used to transfect K12 cells grown in 100-mm-diameter culture dishes. At 28 h after transfection, regular DMEM (−) or DMEM supplemented with 7 μM A23187 (+) was added to the cells. Incubation was continued at 35°C for 16 h. Total cell extracts were prepared and assayed for protein content. Various amounts of protein were used for the CAT assay. The autoradiogram is shown. The amount of protein (in micrograms) used in each enzyme assay and the percentage of chloramphenicol (CM) converted to its 1Ac and 3Ac acetylated forms are indicated. The assay performed with 0.05 U of CAT (P-L Biochemicals) is also shown.

glucose can enhance the intracellular trapping of Ca^{2+} in organelles (1). Our results lead to the intriguing possibility that the cytoplasmic Ca^{2+} concentration may be altered under glucose starvation conditions, resulting in increased transcription of the glucose-regulated genes. Analytical measurement of the intracellular Ca^{2+} concentration under glucose starvation conditions will be a direct test of this hypothesis. Alternatively, glucose starvation may cause other specific effects on cell metabolism which can also trigger an increase in the transcription of these genes. Therefore, it will be useful to determine whether the regulatory elements for the Ca^{2+} and glucose starvation responses can be distinguished.

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