Effects of ketoacidosis on rat apolipoprotein A1 gene expression: a link with acidosis but not with ketones

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ABSTRACT

To determine if ketoacidosis contributes to reduced apolipoprotein A1 (apoA1) expression in insulin-deficient diabetic rats, we examined the regulation of apoA1 gene expression in response to changes in ambient pH or ketone body concentrations. Hepatic apoA1 mRNA levels were reduced 42% in diabetic rats relative to nondiabetic controls (means $\pm$ s.d.; 321.8 ± 43.7 vs 438.7 ± 58.8 arbitrary units; $P < 0.03$). Neither endogenous apoA1 mRNA nor transcriptional activity of the rat apoA1 gene promoter (from -474 to -7) were altered by sodium butyrate or isobutyramide (0.3 mM to 10 mM) in Hep G2 or Caco-2 cells. Rat hepatic and intestinal apoA1 mRNA levels, and plasma apoA1 concentration, were not altered 24 h after isobutyramide administration (500 mg/kg by gavage). When the effect of altering ambient pH within a wide range commonly encountered *in vivo* was

studied, acidosis (pH 6.7), relative to alkalosis (pH 7.9), decreased apoA1 mRNA levels relative to glyceraldehyde-3-phosphate dehydrogenase mRNA by 47% in Hep G2 cells ($P < 0.025$) and by 24% in Caco-2 cells ($P < 0.017$). Acidosis did not alter cytomegalo virus (CMV)- β -galactosidase activity, or the activity of the simian virus (SV40) early-region promoter, in either cell line transfected with the respective constructs. The lowering of ambient pH was associated with a graded reduction in apoA1 promoter activity. At pH 6.7 , apoA1 promoter activity was reduced by 75% compared with promoter activity at pH 7.9 . These observations indicate that acidosis, but not ketosis, contributes to the reduction in apoA1 expression during diabetic ketoacidosis by down-regulating apoA1 promoter activity.

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INTRODUCTION

Apolipoprotein A1 (apoA1) is the major protein constituent of high density lipoprotein (HDL) (Forte & McCall 1994, Barter & Ryek 1996). Epidemiological studies suggest that apoA1 and HDL have cardioprotective properties, presumably because of their roles in reverse cholesterol transport (Miller & Miller 1975, Franceschini *et al.* 1991). Reduced plasma HDL due to inhibition of apoA1 synthesis may contribute to the increased incidence of coronary heart disease in diabetes (Solymoss *et al.* 1995, Despres *et al.* 1996, Mooradian & Nowak 1996, Haffner *et al.* 1998). We recently reported that glucose is a repressor, and

insulin an activator of apoA1 transcription, both *in vitro* and *in vivo* (Murao *et al.* 1998). However, ketoacidosis is another metabolic abnormality commonly found in many subjects with uncontrolled type 1 diabetes. Therefore, we postulated that ketoacidosis may have additional effects on apoA1 gene expression that are independent of changes in plasma glucose and insulin levels. Since ketone bodies (Ortiz-Caro *et al.* 1986, Lazar 1990, deFazio *et al.* 1992, Zitnik *et al.* 1995), as well as acidosis (Yamaji *et al.* 1994, Cassuto *et al.* 1997), independently modulate the expression of several genes, we evaluated the relative roles of each of these parameters in regulating apoA1 gene expression.

MATERIALS AND METHODS

Animals

Male Fischer 344 rats weighing between 200–300 g were purchased from Harlan Laboratories, Indianapolis, IN, USA. The rats were rendered diabetic with a single intraperitoneal injection of 45 mg/kg streptozotocin (Upjohn Co., Kalamazoo, MI, USA), given as a 1.3% solution in citrate buffer (pH 4.5). The rats were killed five days after injection, and the extent of diabetic ketoacidosis was documented by measuring serum glucose concentration using the glucose oxidase method (Beckman glucose analyzer, Beckman Co, Inc., Fullerton, CA, USA), and by measuring serum β -hydroxybutyrate levels using a commercially available kit (Sigma Chemical Co., St Louis, MO, USA). Another group of ten rats was given 500 mg/kg isobutyramide by gavage and killed 24 h later. Isobutyramide, an orally bioavailable butyrate analog, has been shown to stimulate expression of the γ -globin gene promoter *in vivo* (Perrine *et al.* 1994). All rats were killed by exsanguination through the abdominal aorta while under pentobarbital anesthesia (45 mg/kg). The tissues of interest were excised and snap-frozen in liquid nitrogen for RNA extraction.

Cell culture, transfection, and measurement of reporter gene activity

Hep G2 human hepatoblastoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 100 mg/dl glucose, 5% fetal bovine serum (FBS) and penicillin and streptomycin (100 units/ml and 100 g/ml respectively). The human intestinal cell line, Caco-2, was maintained in Earl's modified essential medium (EMEM) supplemented with 15% FBS, non-essential amino acids, 0.11 mg/ml sodium pyruvate and penicillin and streptomycin. Both cell lines were maintained at 37 °C in an atmosphere consisting of 5% CO₂ and 95% air. Cells were transfected with 1 μ g of the plasmid pA1-474.CAT (Romney *et al.* 1992) using lipofectamine (Life Technologies, Inc., Gaithersburg, MD, USA). Construction of the pA1-474.CAT plasmid was reported previously (Romney *et al.* 1992). Briefly, the DNA fragment that spans nucleotides –474 to –7 (relative to the transcriptional start site, +1) of the apoA1 gene was synthesized using the polymerase chain reaction (PCR). Two oligonucleotide primers extending from –474 to –438 and from –32 to –7 were added to the PCR reaction using rat genomic DNA as the template. The PCR-amplified product was purified by agarose gel electrophoresis and inserted

into the SmaI site of the plasmid pT218R (Pharmacia-Amersham Biotech., Piscataway, NJ, USA) for synthesis of single-stranded DNA and sequence verification. The pA1-474.CAT plasmid was then constructed by inserting the apoA1 promoter fragment at the 5'-end of the CAT gene in the vector pUC-CAT. One gram pCMV.SPORT- β -Gal (Life Technologies, Inc.) was added to all transfections to monitor the efficiency of DNA uptake. After 24 h, the cells were treated with either sodium butyrate or isobutyramide, an analog of butyrate, at various concentrations (0, 0.3, 1.0, 3.0, and 10 mM). The viability of cells exposed to butyrate and isobutyramide for 24 h was greater than 90%, as assessed by trypan blue exclusion. To study the effect of acidosis on apoA1 gene promoter activity, Hep G2 and Caco-2 cells were exposed to sodium bicarbonate-depleted DMEM supplemented with 5% FBS, penicillin and streptomycin, and 50 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethane sulfonic acid (HEPES), adjusted to pH 6.7, 7.0, 7.3, 7.6, and 7.9, 24 h after transfection. After another 24 h, cells were harvested and lysed by brief sonication (Branson Ultrasonics Corp., Danbury, CT, USA), and cellular debris was removed by centrifugation at 12 000 $\times g$ for 5 min. To measure β -galactosidase activity, 10 or 20 μ l of the supernatant fraction were incubated in 100 μ l of a solution containing 100 mM sodium phosphate (pH 7.3), 1 mM MgCl₂, 50 mM 2-mercaptoethanol and 0.67 mg/ml *O*-nitrophenyl- β -D-galactopyranoside (Herbomel *et al.* 1984). When color formation was complete, the reaction was terminated by the addition of 150 μ l 1 M sodium carbonate. The reaction was quantified by measuring the absorbance at 420 nm in a multi-plate reader (SLT Lab Instruments, Research Triangle Park, NC, USA). The remaining supernatant fraction was heated at 65 °C for 7 min to inactivate endogenous deacetylase activity, and chloramphenicol acetyltransferase (CAT) activity was measured as described previously (Gorman *et al.* 1982).

Northern blot analysis

Total RNA was isolated from Hep G2 cells, Caco-2 cells, or rat liver and intestine using a single-step acid guanidinium phenol:chloroform extraction procedure (Chirgwin *et al.* 1979). Aliquots of total RNA (10–15 μ g) were separated electrophoretically on a denaturing 1% agarose gel containing 2.2 M formaldehyde (Sambrook *et al.* 1989). The 18S- and 28S-ribosomal RNA bands were visualized by ethidium bromide staining to ensure equivalent loading of total RNA in each lane. The fractionated RNA was transferred to a nylon membrane

FIGURE 1. Expression of hepatic apoA1 mRNA in euglycemic and streptozotocin-diabetic rats. (A) Northern blot of RNA isolated from the livers of vehicle-injected control rats and rats treated with one dose of streptozotocin five days prior to death. A single 0.9 kb band of apoA1 is observed. (B) The mean ($\pm$ s.d.) hepatic apoA1 mRNA relative to G3 PDH mRNA (in arbitrary units) in diabetic and control rats. * $P < 0.027$.

(Hybond, Pharmacia-Amersham Biotech.) and probed with a ^{32}P -labeled human apoA1 cDNA for 2 h at 65 °C in Rapid Hyb (Pharmacia-Amersham Biotech.). After washing the membranes under high-stringency conditions (0.1% sodium dodecyl-sulfate, 0.1 $\times$ standard saline citrate, 65 °C, for 30 min), they were exposed to film for 4–6 h. The

membranes were then stripped as described by the manufacturer and hybridized to a cDNA probe specific for the glyceraldehyde-3-phosphate dehydrogenase (G3 PDH) gene. The amount of hybridization signal was quantified with a scanning laser densitometer (Molecular Dynamics, Sunnyvale, CA, USA).

FIGURE 2. Hepatic apoA1 (top panel) and G3 PDH (bottom panel) mRNA content from control (lanes 1–5) and isobutyramide-treated animals (lanes 6–10). Isobutyramide did not significantly alter hepatic apoAI mRNA content relative to the G3 PDH control.

Western blot analysis

The specificity of the polyclonal apoAI antiserum and the method of its preparation have been described previously (Mooradian *et al.* 1997). Plasma protein samples (5 µg) were electrophoresed in a 10% sodium dodecylsulfate (SDS)-polyacrylamide gel (Laemmli 1970), and transferred electrophoretically to a nitrocellulose membrane (Towbin *et al.* 1979). The membrane was incubated with the apoAI primary antibody at a final dilution of 1:10 000 for 2 h at room temperature. Horseradish peroxidase-linked goat-anti-rabbit IgG was used at a final dilution of 1:10 000 at room temperature. Blots were developed using the enhanced chemiluminescence (ECL) reagent (Pharmacia-Amersham Biotech.) as described by the manufacturer. Plasma apoAI levels were determined by densitometry using the personal densitometer from Molecular Dynamics.

Statistical analysis

Changes in reporter gene activity and mRNA expression were evaluated with the two-tailed

Student's *t*-test. A $P < 0.05$ was considered the limit for statistical significance. The results are expressed as the mean $\pm$ S.D.

RESULTS

Effect of streptozotocin-induced diabetes on apoA1 gene expression

In order to investigate whether or not diabetic ketoacidosis affects apoA1 gene expression, hepatic apoAI mRNA levels, relative to G3 PDH mRNA, were measured in streptozotocin-diabetic rats. The results were compared with age- and sex-matched nondiabetic controls (Fig. 1). All the streptozotocin-treated rats were diabetic since they had blood glucose levels over 300 mg/dl and serum β -hydroxybutyrate levels two- to fourfold above normal. The hepatic apoAI mRNA content of diabetic animals (0.45 ± 0.05 arbitrary units) was only 58% of the apoA1 mRNA found in the euglycemic controls (0.76 ± 0.06 , $P < 0.027$, Fig. 1B).

FIGURE 3. Expression of apoA1 (top panel) and G3 PDH (bottom panel) mRNA in the small intestine from control (lanes 1–5) and isobutyramide-treated animals (lanes 6–10). Isobutyramide treatment did not significantly alter intestinal apoAI mRNA content relative to the G3 PDH control.

Effect of ketone bodies on apoA1 gene expression

In order to determine if ketone bodies affect apoA1 gene expression *in vivo*, four-month-old nondiabetic rats were given 500 mg/kg isobutyramide by gavage. Isobutyramide is an orally bioavailable butyrate analog with long plasma half-life compared with butyrate (Perrine *et al.* 1994). After 24 h isobutyramide treatment, the animals were killed, and RNA was prepared from liver and small intestine. Northern blot analysis of hepatic (Fig. 2) and intestinal (Fig. 3) RNA indicate that isobutyramide has no significant effect on apoA1 mRNA content of these tissues.

Western blot analysis with serum samples obtained from these animals was also performed to determine if ketone bodies have any post-translational effects on steady-state serum apoA1 protein levels. The relative amount of the 29 kDa apoA1 protein did not change in the animals given isobutyramide (data not shown).

Sodium butyrate (10 mM) and isobutyramide (10 mM) did not alter apoAI mRNA levels in Hep G2 or Caco-2 cells in culture (data not shown).

These results suggest that ketone bodies, *per se*, do not have a readily demonstrable effect on the steady-state levels of the apoA1 message in either cultured Hep G2 or Caco-2 cells or *in vivo*.

Effect of ketone bodies on the apoA1 gene promoter

To determine if ketone bodies have any effects on apoAI gene transcriptional activity, reporter gene expression driven by the apoAI promoter was analyzed in cultured cells. Hep G2 liver cells and Caco-2 intestinal cells were transfected with the plasmid pA1-474.CAT, which carries the full sequence of rat apoAI gene promoter, along with the CAT reporter gene (Widom *et al.* 1991, Romney *et al.* 1992, Taylor *et al.* 1996, Mooradian *et al.* 1997, Harnish *et al.* 1998, Murao *et al.* 1998). Both of these cell lines are suitable for these studies since they express the endogenous apoA1 gene (see below). After 24 h, the cells were treated with either sodium butyrate (Btr) and isobutyramide (Iso) at concentrations of 0.3, 1.0, 3.0, and 10.0 mM. After 24 h, the cells were harvested and assayed for CAT

TABLE 1. ApoAI promotor activity (CAT activity) in the presence of varying concentrations of butyrate and isobutyramide. Results are means ± the variation

Concentration (mM)	CAT activity* (% acetylation)	
	Butyrate	Isobutyramide
0	16.8 ± 0.0	16.8 ± 0.0
0.3	13.0 ± 0.0	16.5 ± 2.5
1.0	18.4 ± 4.8	17.7 ± 1.6
3.0	14.7 ± 1.5	14.3 ± 2.4
10.0	14.0 ± 4.1	16.7 ± 1.2

*CAT activity normalized to expression of β-galactosidase activity. No significant dose-responent relationship is observed in the duplicate measurements. These findings were confirmed in similar experiments, repeated twice.

and β-galactosidase activity. The results (Table 1) show that Btr or Iso did not significantly alter CAT activity relative to β-galactosidase activity, at any dose. The activity of the CMV-β-gal reporter gene used to control for transfection efficiency was not altered when Btr or Iso were added to culture media.

Effect of pH on apoAI mRNA levels

Since the presence of ketone bodies in the absence of changes in culture media pH did not affect expression of the apoAI gene, we next determined whether or not acidosis affects apoAI mRNA levels *in vitro*. Total RNA was isolated from both Hep G2 and Caco-2 cells maintained at either pH 6.7 or 7.9 for 24 h, and Northern blotting was performed with probes specific for the apoAI and G3PDH mRNAs. The results (Fig. 4) indicate that apoAI mRNA content is reduced 47% and 24% during acidosis in Hep G2 ($P<0.025$, Fig. 4A) and Caco-2 ($P<0.017$, Fig. 4B) cells respectively. G3PDH mRNA levels were similar at pH 6.7 and pH 7.9, suggesting that the effect of pH on apoAI mRNA content is specific.

Effect of pH on the apoA1 gene promoter

Since apoAI mRNA levels are suppressed by acidosis, we determined whether or not the effect of acidosis is mediated through the apoA1 gene promoter. Hep G2 and Caco-2 cells were transfected with the reporter construct pA1.474.CAT. After 24 h, the media were removed and replaced with bicarbonate-deficient DMEM maintained at the required pH using HEPES buffer. After 24 h, the cells were collected and assayed for CAT and β-galactosidase activity. In Hep G2 cells, a

A. Hep G2

B. Caco-2

FIGURE 4. Effect of acidosis on apoAI mRNA. Total RNA was isolated from both Hep G2 (A) and Caco-2 (B) cells maintained at either pH 6.7 (lanes 1–3) or pH 7.9 (lanes 4–6) for 24 h. The membrane was hybridized with the rat apoAI cDNA, stripped, and rehybridized with the G3PDH cDNA. The amount of signal generated with the apoAI probe was normalized to the amount obtained with the G3PDH probe. The increase in apoAI mRNA (0.9 kb) relative to G3PDH mRNA (1.2 kb) is evident at pH 7.9 compared with pH 6.7.

reduction in extracellular pH from 7.9 to 6.7 gradually decreased CAT activity more than fourfold (Fig. 5 and Table 2). A similar trend was also observed in Caco-2 cells (Fig. 6 and Table 2).

FIGURE 5. ApoA1 gene promoter activity in Hep G2 cells cultured in media with varying pH. Hep G2 cells were transfected with pA1-474.CAT (full-length apoA1 promoter) and after 24 h, the media were removed and replaced with growth media adjusted to pH 6.7, 7.0, 7.3, 7.6, and 7.9. Control media (C) contain sodium bicarbonate. The sagging appearance of the acetylated products is occasionally observed when large TLC plates are used. Reduced ^{14}C -chloramphenicol acetylation at pH 6.7 compared with higher pH is evident. The quantitative results are presented in Table 2. N, nontransfected cells; C, cells cultured in bicarbonate-containing media.

The activity of the cytomegalo virus (CMV)- β -gal control plasmid was reduced in some experiments 20–25% at the lowest pH, but did not change from pH 7.0 to 7.9 (data not shown). This slight decrease in β -galactosidase activity may be due to the effect of an acidic environment on protein synthesis in some cell lines (Yamaji *et al.* 1994). In order to determine if the change in apoAI promoter activity we observed with acidosis is not an artifact of the assay or culture conditions, we transfected Caco-2 cells with the plasmid pSV₂CAT, which contains the simian virus 40 (SV40) early-region promoter fused to the CAT reporter gene. After 24 h, the media were adjusted to either pH 6.7 or 7.9, and after a further 24 h, the cells were harvested and assayed for CAT

TABLE 2. Effect of varying ambient pH on apoAI promoter activity (CAT activity) in Hep G2 and Caco-2 cells. Results are means $\pm$ the variation

pH	CAT activity* (% acetylation)	
	Hep G2	Caco-2
6.7	2.1 $\pm$ 0.2	12.1 $\pm$ 0.1
7.0	4.0 $\pm$ 0.2 [†]	13.4 $\pm$ 1.8
7.3	4.8 $\pm$ 0.1 [†]	17.1 $\pm$ 0.1 [†]
7.6	6.1 $\pm$ 0.6 [†]	18.8 $\pm$ 7.7 [†]
7.9	8.1 $\pm$ 0.3 [†]	31.0 $\pm$ 6.3 [†]

*CAT activity is normalized to β -galactosidase activity.

[†] $P < 0.05$ compared to the measurements at pH 6.7.

The graded increase in CAT activity with increasing pH is evident in both cell lines.

FIGURE 6. ApoA1 gene promoter activity in Caco-2 cells cultured in media with varying pH. Caco-2 cells were transfected with pA1-474.CAT (full-length apoA1 promoter) and after 24 h, the media were removed and replaced with growth media adjusted to pH 6.7, 7.0, 7.3, 7.6, and 7.9. Control media (C) contain sodium bicarbonate. Reduced ^{14}C -chloramphenicol acetylation at pH 6.7 compared with higher pH is evident. The quantitative results are presented in Table 2. N, nontransfected cells; C, cells cultured in bicarbonate-containing media.

activity. The results (Fig. 7) indicate that the SV40 early-region promoter activity is not significantly altered by changing the ambient pH. These data support the conclusion that the rat apoA1 gene promoter, unlike the SV40 early-region promoter, is sensitive to changes in extracellular pH.

DISCUSSION

The association of heart disease with diabetes has been reported in several epidemiological studies and requires an explanation at the molecular level. Glucose and insulin regulate expression of the apoA1 gene in the liver (Murao *et al.* 1998) through an insulin response core element (IRCE) located in

the 5'-flanking region of the rat apoA1 gene. The IRCE present in the apoAI promoter is related to the IRCEs identified in the G3PDH gene and glucagon gene (Nasrin *et al.* 1990, Philippe 1991). This process may have a role in reducing apoA1 mRNA expression in diabetic animals (Fig. 1).

Since four-month-old rats with severe streptozotocin-induced diabetes have low hepatic apoAI mRNA levels (Fig. 1), we wished to determine if, in addition to hyperglycemia and insulin deficiency, ketoacidosis also affects expression of the apoA1 gene in the liver and small intestine. Small chain fatty acids, such as sodium butyrate, are structurally similar to the ketone bodies that are elevated in diabetic ketoacidosis. Sodium butyrate regulates gene expression (Ortiz-Caro *et al.* 1986, Lazar 1990, deFazio *et al.*

pH: 6.7 7.9

CAT Activity: 83.1 73.2
±3.3 ±3.9

FIGURE 7. Caco-2 cells were transfected with pSV₂CAT and after 24 h, the media were exchanged with HEPES-buffered media at either pH 6.7 or 7.9. After another 24 h, cells were harvested and assayed for CAT activity. The change in media pH did not significantly alter CAT activity when normalized to β -galactosidase activity.

1992, Zitnik *et al.* 1995) by enhancing histone acetylation through inhibition of histone deacetylase activity (Turner 1993), by activating phosphatase activity (Cuisset *et al.* 1997), or by other means that await discovery. The present studies evaluating the ability of butyrate to modulate expression of the rat apoA1 gene through its promoter *in vitro*, did not show any change in CAT activity relative to untreated controls (Table 1). Isobutyramide (Iso), a derivative of Btr with a longer plasma half-life *in vivo* (Perrine *et al.* 1994), also had no effect on CAT activity in transfected liver cells (Table 1). In addition, there were no changes in apoA1 mRNA or protein content in either the liver or small intestine

of animals treated with Iso (Figs 2 and 3), suggesting that our *in vitro* studies are consistent with the *in vivo* data. These observations suggest that ketone bodies, *per se*, have no demonstrable effect on the expression of the apoA1 gene.

In contrast to the lack of a demonstrable effect of ketone bodies on apoAI mRNA content or apoAI promoter activity, our experiments demonstrate that apoA1 expression is extremely sensitive to variations in extracellular pH. This effect is observed in both the liver and intestinal cell lines (Figs 5 and 6, and Table 2), suggesting that the signal elicited by the change in pH is conserved, and most likely mediated, by a mechanism common to both cell lineages. ApoAI gene expression in the region of the small intestine proximal to the duodenum may be sensitive to acidosis during the daily excursions in pH associated with feeding and the emptying of stomach contents. The resulting acidic environment is transient due to the rapid activation of bicarbonate/chloride pumps located in the intestinal epithelial cell. Whether or not bicarbonate ions affect expression of the apoAI gene remains to be examined. An acidic extracellular pH is sometimes associated with decreases in protein synthesis in some cell lines (Yamaji *et al.* 1994). We occasionally observed a similar phenomenon in some of our experiments when assessing both CAT and β -galactosidase activity at pH 6.7, the lowest value we examined. Experiments with the SV40 virus early-region promoter (Fig. 7), however, indicate that the activity of this promoter at pH 6.7 and pH 7.9 is equivalent. Furthermore, the relative specificity of our findings is also indicated by the observation that apoAI mRNA levels were reduced relative to the G3PDH mRNA (Fig. 4).

The acidosis that occurs in ketotic animals has the potential of affecting the expression of various genes. Previously published studies have shown that several genes in the kidney respond at the transcriptional level to changes in pH (Yamaji *et al.* 1994). The activity of the transcription factor early growth response factor one (EGR-1), as well as mRNA levels for several transcription factors involved in immediate-early gene expression, are elevated by a protein tyrosine kinase-dependent pathway when kidney epithelial cells are exposed to an acidic environment (Yamaji *et al.* 1994). Tyrosine kinase inhibitors substantially reduce the effect of acidosis on transcription, but the actual kinases involved in the signal transduction cascade remain to be elucidated. Interestingly, the apoAI gene promoter has an EGR-1 binding site (Kilbourne *et al.* 1995) which may be responsible for the pH effect described here. Acidosis may

enhance the ability of EGR-1 to bind the apoA1 promoter and either act as a transcriptional repressor or prevent the activity of another transcription inducing factor. Alternatively, acidosis may affect the tertiary structure of the apoA1 promoter. The rate of base depurination as well as strand dissociation are enhanced in an acidic environment. Unless the apoA1 promoter has a region that is particularly sensitive to these changes, this effect would also most likely affect the expression of other genes. It is more likely that the effect of acidosis is mediated by a *trans*-acting repressor for two reasons. First, repression by acidosis is specific for the apoA1 promoter. The SV40 virus early-region promoter was not significantly repressed when the pH was dropped to pH 6.7 (Fig. 7). Secondly, a slight reduction in reporter gene activity is evident with each gradual change in pH. The lack of a threshold phenomenon suggests that an intracellular pH 'sensor' is gradually engaged when the pH is altered (Figs 5 and 6). Experiments to characterize the *cis*-element(s) involved in regulating apoA1 gene expression by acidosis, as well as characterizing the roles of tyrosine kinases and protein kinase C on mediating the affect of acidosis on apoA1 gene expression, are currently under way.

In summary, these observations indicate that ketoacidosis suppresses apoA1 gene expression. The effect is solely the result of acidemia and is not related to increased ketone body concentration. These changes in apoA1 mRNA synthesis may contribute to the high incidence of coronary atherosclerotic heart disease in type 1 diabetic subjects and to the decreased apoA1 level observed in children with chronic renal failure and metabolic acidosis (Bircan *et al.* 1997).

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