20. No enhancement of HIV-1 LTR transactivation was observed with another human CD4+ T cell line (CEM_{50}) persistently infected with X-MuLV in vitro.
21. The CD4+ T cell line CEM_{50} was persistently infected with a well-characterized MuLV-like amphotropic retrovirus (A-MuLV) grown in mink cell monolayers [M. L. Bryant and V. Klement, *Virology* **73**, 532 (1976); S. Rasheed, M. B. Gartner, E. Chan, *J. Virol.* **19**, 13 (1976); J. W. Hartley and W. P. Rowe, *ibid.*, p. 19]. The amphotropic nature of this virus was confirmed by its ability to productively infect cells of both mouse and human origin. Exposure of the CEM_{50}/A-MuLV cell line to HIV-1 (strain $HTLV\text{-}III_B$) lead to productive coinfection with extensive cytopathic effect in 4 to 6 days.
22. B. H. Hahn *et al.*, *Nature* **312**, 166 (1984).
23. A. S. Huang *et al.*, *J. AIDS*, in press.
24. R. Carr *et al.*, *Lancet* **i**, 1431 (1984); G. Sieber *et al.*, *Blut* **51**, 143 (1985).
25. R. W. Schroff *et al.*, *Clin. Immunol. Immunopathol.* **27**, 300 (1983); J. L. Fahey *et al.*, *Am. J. Med.* **76**, 95 (1984).
26. R. Snyderman and G. J. Cianciolo, *Immunol. Today* **5**, 240 (1984); G. J. Cianciolo, T. D. Copeland, S. Oroszlan, R. Snyderman, *Science* **230**, 453 (1985).
27. H. G. Bedigian, R. R. Fox, H. Meier, *Cancer Res.* **36**, 4693 (1976); *J. Virol.* **27**, 313 (1978).
28. L. Bacheler and H. Fan, *J. Virol.* **37**, 181 (1981).
29. T. Lea *et al.*, *Scand. J. Immunol.* **22**, 207 (1985).
30. V. Kalyanaraman, R. Pal, R. C. Gallo, M. G. Sarngadharan, *AIDS Res. Hum. Retroviruses* **4**, 319 (1989).
31. W. M. Hunter and F. C. Greenwood, *Nature* **194**, 495 (1962).
32. F. D. Veronese, T. D. Copeland, S. Oroszlan, R. C. Gallo, M. G. Sarngadharan, *J. Virol.* **62**, 795 (1988).
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Glucose, Sulfonylureas, and Neurotransmitter Release: Role of ATP-Sensitive K^+ Channels

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Sulfonylurea-sensitive adenosine triphosphate (ATP)–regulated potassium (K_{ATP}) channels are present in brain cells and play a role in neurosecretion at nerve terminals. K_{ATP} channels in substantia nigra, a brain region that shows high sulfonylurea binding, are inactivated by high glucose concentrations and by antidiabetic sulfonylureas and are activated by ATP depletion and anoxia. K_{ATP} channel inhibition leads to activation of γ-aminobutyric acid (GABA) release, whereas K_{ATP} channel activation leads to inhibition of GABA release. These channels may be involved in the response of the brain to hyper- and hypoglycemia (in diabetes) and ischemia or anoxia.

ATP-DEPENDENT POTASSIUM CHANnels are critical for insulin secretion from pancreatic β-cells (*1–3*). They are inhibited by glucose, which in turn leads to insulin secretion (*2, 3*), and activated by polypeptide hormones such as galanin and somatostatin (*4–6*), which are known inhibitors of insulin release. K_{ATP} channels are the target of an important class of antidiabetic drugs, the sulfonylureas (*2, 3, 7, 8*). These channels open and produce a hyperpolarization when the intracellular concentration of ATP ($[ATP]_{in}$) decreases, and close and lead to depolarizations when $[ATP]_{in}$ increases (*2, 3*). Glucose, as well as the sulfonylureas, induces a depolarization, which then leads to the activation of L-type Ca^{2+} channels, to Ca^{2+} entry, and to insulin secretion. The depolarizing step is due to direct (sulfonylureas) or indirect (glucose, probably by an increase of $[ATP]_{in}$) blockade of K_{ATP} channels.

The central nervous system is a rich source of sulfonylurea receptors (*9*). These receptors are present in high concentrations in different areas of the brain (*10*); one of the predominant areas is the substantia nigra (SN), in which the cells contain as many K_{ATP} channels as pancreatic β-cells (*7, 10*). We have investigated the role of these channels in the release of GABA, which is a key neurotransmitter in SN (*11*).

Secretion of GABA from SN slices (*12*) is similar to secretion of insulin from pancreatic cells. [^{3}H]GABA release is stimulated by K^+ depolarization (Fig. 1A) or exposure to glucose (Fig. 1B) or to the potent sulfonylurea gliquidone (Fig. 1C) in a dose-dependent manner. [^{3}H]GABA release is stimulated to a similar extent by 20 m*M* glucose and 100 n*M* gliquidone. Half-maximal stimulation of [^{3}H]GABA release by gliquidone (EC_{50}) is observed at 3 n*M*. We also compared the efficacy of a series of hypoglycemic sulfonylureas (Fig. 1D). Half-maximal stimulatory effects vary between 3 n*M* and 300 μ*M*; the most active compound was gliquidone and the least active was tolbutamide.

K_{ATP} channels are permeable to both K^+ and Rb^+. $^{86}Rb^+$ efflux (*13*) has been useful for identifying K_{ATP} channels in insulinoma cells and for establishing their basic pharmacological and regulatory properties (*4, 6, 7*). As is true in insulinoma cells, in SN a mixture of oligomycin and 2-deoxyglucose (2DG) inhibits ATP formation from both

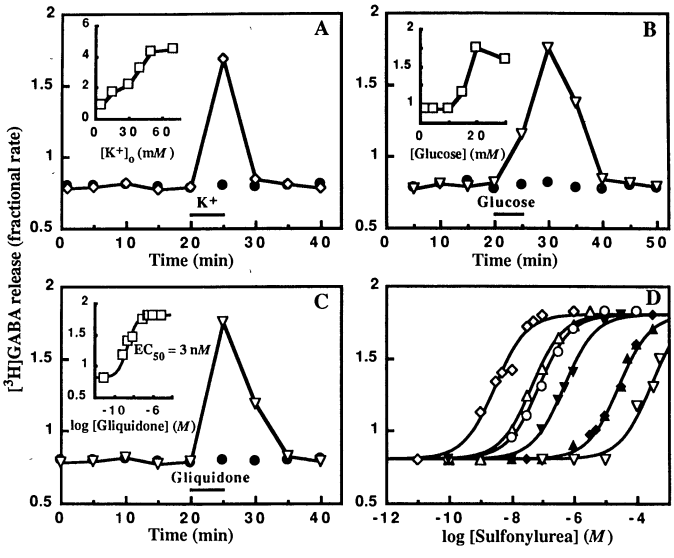

Fig. 1. [^{3}H]GABA-evoked release by (**A**) extracellular potassium concentration $[K^+]_o$, (**B**) glucose, and (**C**) gliquidone. Horizontal bars represent the period of stimulation by effectors. (A) Control in medium (●), activation by 15 m*M* K^+ (◇). Inset, dose-response curve for K^+ activation (at 5 min). To keep ionic strength constant, we modified the medium so that $[Na^+]_o$ plus $[K^+]_o$ was 123.5 m*M*. (B) Control in medium (●), activation by 20 m*M* glucose (▽). Inset, dose-response curve for glucose activation (at 10 min). (C) Control (●), activation by 100 n*M* gliquidone (▽). Inset, dose-response curve for gliquidone activation (at 5 min). (**D**) Release of [^{3}H]GABA was evoked by increasing concentrations of gliquidone (◇) (EC_{50}, 3 n*M*), LH35 (△) (EC_{50}, 50 n*M*), glipizide (○) (EC_{50}, 80 n*M*), LH32 (▼) (EC_{50}, 500 n*M*), glibenclamide (▲) (EC_{50}, 27.5 μ*M*), glisoxepide (◆) (EC_{50}, 27.5 μ*M*), and tolbutamide (▽) (EC_{50}, 300 μ*M*). Data points represent the means of four experiments.

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oxidative phosphorylation and glycolysis and leads to a decrease in $[ATP]_{in}$ in SN slices (Fig. 2A, inset). This treatment triggers a $^{86}Rb^+$ efflux from SN that is completely inhibited by gliquidone (100 n*M*) (Fig. 2A), as also occurs in insulinoma cells. $^{86}Rb^+$ efflux is probably occurring through K_{ATP} channels that have been opened by the $[ATP]_{in}$ depletion. The relative potency of different sulfonylureas in blocking these channels is shown in Fig. 2C.

Pathological situations such as anoxia or ischemia decrease $[ATP]_{in}$ in brain tissue. Indeed replacement of the normal oxygenated medium (containing O_2, CO_2, and glucose) by the anoxic medium (containing N_2, CO_2, and glucose) decreases $[ATP]_{in}$ in SN slices (Fig. 2B, inset) and triggers a parallel $^{86}Rb^+$ efflux that is completely blocked in the presence of gliquidone (Fig. 2B). The efficacy of anoxia in provoking the gliquidone-sensitive $^{86}Rb^+$ efflux is linked to the extracellular glucose concentration. Exposure to anoxia in the presence of 20 m*M* instead of 10 m*M* glucose nearly abolishes the gliquidone-sensitive $^{86}Rb^+$ efflux component produced by anoxia (Fig. 2D). The different sulfonylureas block nearly identically $^{86}Rb^+$ efflux induced either by treatment with oligomycin plus 2DG or by anoxia (Fig. 2C).

Finally, there is a linear relation (Fig. 3) between the potency of different sulfonylureas in blocking $^{86}Rb^+$ efflux in response to poisoning and in stimulating [^{3}H]GABA release. The most active sulfonylurea in inhibiting $^{86}Rb^+$ efflux in SN is gliquidone, whereas it is glibenclamide in insulinoma cells (*7*). Thus, the sulfonylurea receptors appear to be similar, but not identical, in SN and pancreatic β-cells.

All these results together with our previous data demonstrating high concentrations of sulfonylurea receptors in the SN (particularly in pars reticulata) (*11*) show that there are K_{ATP} channels in SN, that they are inhibited by sulfonylureas and inactivated by elevated glucose concentrations, and that they play a central role in the control of GABA release from SN terminals that in turn controls the activity of dopamine-containing neurons.

High levels of blood glucose suppress the firing of dopamine-containing neurons in SN (*14*). Our interpretation of this effect of glucose is that high glucose concentrations close K_{ATP} channels in terminals from neurons in SN that innervate dopamine-containing neurons. This inhibitory effect leads to depolarization of GABA-containing terminals then to GABA release and subsequent inhibition of dopamine-containing neurons.

SN is a central gating system for generalized convulsive activity, and GABA in SN plays a critical role in seizure control (*15–17*). A critical level of nerve terminal GABA must be maintained in SN to protect against generalized seizures, although these seizures do not originate from the SN. Diabetic patients tend to develop seizures in the course of hypoglycemia. Our results suggest that a decrease in blood glucose will decrease GABA release from SN nerve terminals through hyperpolarization due to the opening of K_{ATP} channels. It is tempting to speculate that this decrease in the inhibitory capacity of the GABA system during hypoglycemia will eliminate seizure protection by SN. For such a speculation to be correct, one would need to have a dose-response curve of the glucose effect on GABA release in vivo in a physiological range of glucose concentration between 2 to 3 m*M* and 10 m*M* glucose (instead of 10 and 20 m*M* in the in vitro conditions of Fig. 1B).

A decrease of the inhibitory capacity of the GABA system is also to be expected in anoxia (and in ischemia), which decreases $[ATP]_{in}$ and opens K_{ATP} channels. The development of seizures is a complication of cerebral ischemia (*15*).

Status epilepticus, which appears to be linked to low GABA levels in SN (*18*), causes lesions of SN; ischemia that is associated with a decrease of $[ATP]_{in}$ and an inhibition of GABA release (Fig. 2) also produces SN damage (*16*). GABA is essential to protect SN against delayed transneuronal death after destruction of caudate nucleus (*19*).

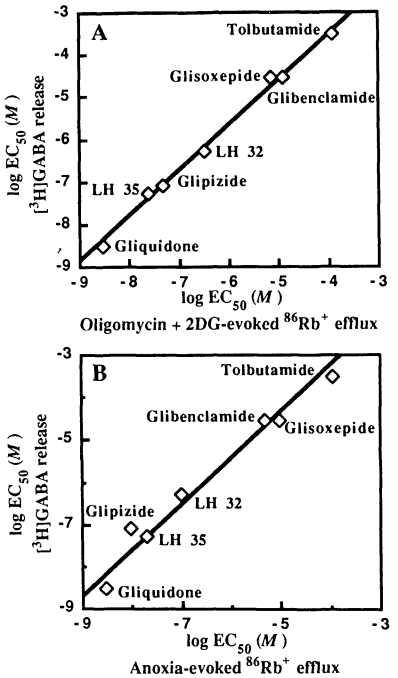

Fig. 3. Structure-function relations for the effect of sulfonylureas on $^{86}Rb^+$ efflux and inhibition of GABA release. EC_{50} values of different sulfonylureas for activation of [^{3}H]GABA release were plotted as a function of EC_{50} values of the same compounds for inhibition of $^{86}Rb^+$ efflux activated (**A**) by oligomycin plus 2DG treatment (slope of line = 1.08 ± 0.03) or (**B**) by anoxia (slope of line = 1.02 ± 0.08).

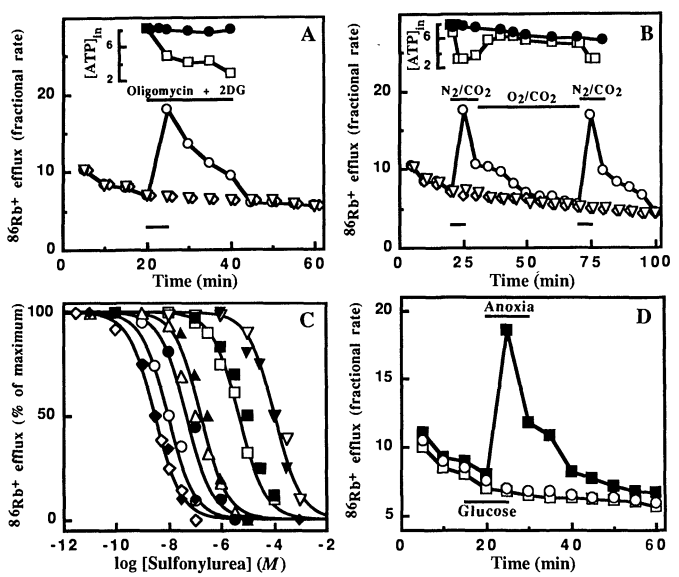

Fig. 2. (**A**) Kinetics of $^{86}Rb^+$ efflux from SN slices, control (▽) in the presence of oligomycin plus 2DG without (○) and with 100 n*M* gliquidone (◇) (lower bar). Inset, time dependence of $[ATP]_{in}$ in the absence (●) or in the presence of oligomycin plus 2DG (□). (**B**) Kinetics of $^{86}Rb^+$ efflux without (▽) or with anoxia in the absence (○) or the presence of 100 n*M* gliquidone (◇) (lower bar). Inset, variation of $[ATP]_{in}$ in control (●) or in anoxic conditions (□). In both insets, $[ATP]_{in}$ is in nanomoles per milligram of protein. Data points represent the means of $n = 8$. (**C**) Activated $^{86}Rb^+$, efflux after 5 min in oligomycin plus 2DG (closed symbols) or in anoxic conditions (open symbols), is inhibited by increasing concentrations of gliquidone (◇, ◆), glipizide (○, ●), LH32 (△, ▲), glibenclamide (□, ■), and tolbutamide (▽, ▼). Results with LH35 and glisoxepide are not shown (see Fig. 3). (**D**) Kinetics of $^{86}Rb^+$ efflux from SN slices without (□) or with anoxia (■), carried out in 10 m*M* (■) or in 20 m*M* glucose (○). Data points represent the means of $n = 6$ to 8. Unlike the results in β-cells, glucose does not reduce basal $^{86}Rb^+$ efflux, nor do the sulfonylureas. This may be a technical problem, that is, there are too few channels to be detected without stimulation, or there is a larger proportion of contaminating, nonsensitive cells in the preparation that give a higher background.

Increases in blood glucose levels also have pathological effects that seem to be strongly correlated to SN and have potentially important implications for diabetes. The locomotor activity of experimental animals is closely related to the activity of dopamine-containing neurons (*20*). Yet, the ambulatory activity of streptozotocin-induced diabetic rats is decreased when the blood glucose level rises (*21*). We would like to propose that high glucose levels inhibit K_{ATP} channels, depolarize SN terminals, increase GABA release, and thereby also extensively inhibit the activity of dopaminergic neurons (*21*), resulting in a decrease of ambulatory activity.

REFERENCES AND NOTES

1. P. R. Stanfield, *Trends Neurosci.* **10**, 335 (1987).
2. O. H. Petersen and I. Findlay, *Physiol. Rev.* **67**, 1054 (1987).
3. F. M. Ashcroft, *Annu. Rev. Neurosci.* **11**, 97 (1988).
4. J. R. De Weille, H. Schmid-Antomarchi, M. Fosset, M. Lazdunski, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 1312 (1988).
5. M. J. Dunne *et al.*, *EMBO J.* **8**, 413 (1989).
6. J. R. De Weille, H. Schmid-Antomarchi, M. Fosset, M. Lazdunski, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2971 (1989).
7. H. Schmid-Antomarchi, J. R. De Weille, M. Fosset, M. Lazdunski, *J. Biol. Chem.* **262**, 15840 (1987).
8. N. C. Sturgess, M. L. J. Ashford, D. L. Cook, C. N. Hales, *Lancet* **ii**, 474 (1985).
9. H. Bernardi, M. Fosset, M. Lazdunski, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 9816 (1988).
10. C. Mourre, Y. Ben Ari, H. Bernardi, M. Fosset, M. Lazdunski, *Brain Res.* **486**, 159 (1989).
11. J. H. Fallon and S. E. Loughlin, in *The Rat Nervous System*, G. Paxinos, Ed. (Academic Press, San Diego, 1985), vol. 1, pp. 353–374.
12. Wistar rats (200 g) were killed by decapitation, and their brains were rapidly removed. SN slices (0.7 mm) were dissected with a McIlwain chopper and rapidly incubated in medium previously gassed with 95% O_2 and 5% CO_2 and containing 120 m*M* NaCl, 3.5 m*M* KCl, 1 m*M* $MgSO_4$, 16 m*M* $NaHCO_3$, 1.2 m*M* $CaCl_2$, 10 m*M* D-glucose in 11 m*M* Hepes-NaOH buffer (*p*H 7.4) at 37°C. Slices were gassed for 90 min before the beginning of experiments in order to permit a full recovery. Aeration was continuous during the whole procedure. Slices were preloaded for 20 min with 1 μ*M* [^{3}H]GABA (2 μCi/ml) in 1 ml of medium in the presence of 50 μ*M* aminooxyacetic acid to inhibit GABA transaminase. A 45-min washing was done before beginning the release experiments. Then one slice was transferred per well of a multiwell box (1 ml of oxygenated medium per well). [D. Minc-Golomb, Y. Levy, N. Kleinberger, M. Schramm, *Brain Res.* **402**, 255 (1987)]. Release experiments were then carried out during consecutive intervals of 5 min for 40 to 50 min. Eight independent experiments were performed at the same time. Fractional rates of release were calculated as [^{3}H]GABA released during each 5-min interval and expressed as the percentage of the [^{3}H]GABA content in the tissue at the beginning of the respective intervals.
13. Slices were loaded for 30 min with 5 to 10 μCi/ml of $^{86}Rb^+$. A 30-min washing was carried out before the $^{86}Rb^+$ efflux was started. Experiments were then performed as for [^{3}H]GABA release. Effectors were added as described in the figures. For anoxic conditions we used 95% N_2/5% CO_2 instead of 95% O_2/5% CO_2; 10 m*M* glucose was used in each case. Intracellular ATP depletion was obtained when necessary by adding oligomycin (2.4 μg/ml) and 1 m*M* 2DG (*8*) in incubation medium without glucose and in O_2/CO_2 conditions. After solubilizing the slices with 1% Triton X-100, total $[ATP]_{in}$ was measured with the luciferase-luciferin technique [R. Christen, R. W. Schackman, B. M. Shapiro, *J. Biol. Chem.* **258**, 5392 (1983)]. Proteins were measured [M. M. Bradford, *Anal. Biochem.* **72**, 248 (1976)] with bovine serum albumin as standard.
14. C. F. Saller and L. A. Chiodo, *Science* **210**, 1269 (1980).
15. M.-L. Smith, H. Kalimo, D. S. Warner, B. K. Siesjö, *Acta Neuropathol.* **76**, 253 (1988).
16. K. Gale, *Fed. Proc.* **44**, 2414 (1985).
17. ———, *Adv. Neurol.* **44**, 343 (1986).
18. G. Nevander, M. Ingvar, R. N. Auer, B. K. Siesjö, *Ann. Neurol.* **18**, 281 (1985).
19. M. Saji and D. J. Reis, *Science* **235**, 66 (1987).
20. D. C. Hoffman and R. J. Beniger, *Pharmacol. Biochem. Behav.* **22**, 341 (1985).
21. Y. Shimomura *et al.*, *Endocrinology* **123**, 2621 (1988).

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Functional Properties of Rat Brain Sodium Channels Expressed in a Somatic Cell Line

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Transfection of Chinese hamster ovary cells with complementary DNA encoding the R_{IIA} sodium channel α subunit from rat brain led to expression of functional sodium channels with the rapid, voltage-dependent activation and inactivation characteristic of sodium channels in brain neurons. The sodium currents mediated by these transfected channels were inhibited by tetrodotoxin, persistently activated by veratridine, and prolonged by *Leiurus* α-scorpion toxin, indicating that neurotoxin receptor sites 1 through 3 were present in functional form. The R_{IIA} sodium channel α subunit cDNA alone is sufficient for stable expression of functional sodium channels with the expected kinetic and pharmacological properties in mammalian somatic cells.

SODIUM CHANNELS MEDIATE THE MAJOR inward current responsible for the upstroke of the action potential in many excitable cells. Rat brain Na^+ channels are heterotrimeric proteins consisting of α, β_1, and β_2 subunits of 260, 36, and 33 kD, respectively (*1*). A wide variety of neurotoxins affect Na^+ channel activity, and five different toxin receptor sites on the rat brain channel have been biochemically characterized (*1*). Three distinct subtypes of the rat brain Na^+ channel α subunit, R_I, R_{II}, and R_{III}, have been cloned and sequenced (*2, 3*). Expression of α subunits by injection of mRNA into *Xenopus* oocytes has shown that many Na^+ channel functions are mediated by the α subunit alone (*4–7*). However, high molecular weight brain mRNA or R_{IIA} mRNA direct the synthesis of Na^+ channels with unusually slow inactivation kinetics (*7*). Coexpression with size-fractionated brain mRNA smaller than 4 kb is able to accelerate inactivation, suggesting that a low molecular weight brain protein is necessary for rapid inactivation in oocytes (*7*). We have stably expressed cDNA for the R_{IIA} α subunit in Chinese hamster ovary (CHO) cells, a mammalian cell line that lacks endogenous voltage-sensitive Na^+ channels, in order to examine the physiological and pharmacological properties of these channels in the genetic background of a mammalian somatic cell.

CHO cells were cotransfected (*8*) with pVA222 containing the coding region of the R_{IIA} Na^+ channel α subunit (*7*) in vector pECE (*9*) and pSV2neo, a plasmid conferring resistance to the antibiotic G418. Control transfections with pECE lacking the α subunit were also performed. Transfectants were selected through multiple passages in the presence of G418 (*8*). Seven independent stable G418-resistant cell lines were isolated and analyzed for the presence of the α subunit transcript by the ribonuclease (RNase) protection procedure. Cell lines PVA1 and PVA4 expressed RNA that protected a 781-nucleotide (nt) cRNA antisense transcript covering nucleotides 50 to 831 of the R_{IIA} 5′ region (*7*) (Fig. 1A). Northern (RNA) blots showed that these two cell lines expressed mRNA species of approximately 7.5 and 3.5 kb, which were specifically recognized by an R_{IIA} cRNA probe (Fig. 1B, lanes 3 and 4). Although 7.5 kb is the expected size for transcription of the R_{IIA} cDNA in the pECE vector, the 3.5-kb species is truncated, possibly due to premature termination of transcription or

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