is likely that Rev has several contact points within the RRE. Our previous observation that formation of the large stem structure is required for Rev interaction (10), taken together with the findings reported here, suggests that Rev has contact points on both the large stem and the HSL-5 hairpin loop that are in close proximity. By deletion analysis of the RRE, Dayton et al. (16) have also found that maintenance of secondary structure is required for Rev response. In other experiments with the RRE elements obtained from the evolutionarily conserved simian immunodeficiency virus (SIV) and HIV-2 viruses, we find that both interact with the HIV-1 Rev protein (17). However, the hairpin loop structures required for this interaction, which are also in close proximity to the stem structure, bear little sequence similarity to the HIV-1 hairpin loop recognized by Rev. This observation strengthens our contention that structure, rather than simply primary sequence, determines Rev-RRE interactions. This may also explain how the human T cell lymphotropic virus type 1 (HTLV-1) Rex protein, which is unrelated to HIV-1 Rev, can substitute for Rev function (18). Furthermore, the strong correlation between the alteration of the binding in vitro and the functional characteristics of the mutant RREs in vivo supports the hypothesis that binding of Rev to the RRE is of physiological relevance.

The mechanisms by which Rev-RRE interactions facilitate export of HIV structural mRNAs from the nucleus to cytoplasm remain to be determined. Whether Rev acts by itself or in concert with host proteins to facilitate export is not known. We have recently purified a transdominant Rev protein described by Malim et al. (19). Although nonfunctional in vivo, as reported, it does form a stable complex with the RRE (20). Thus, if Rev mediates export through interaction with a host transporter protein, the transdominant Rev is likely to lack this function. As an alternative explanation, the interaction of Rev with the RRE may facilitate a conformational change in the RRE secondary or tertiary structure, which permits access of a host "transporter" factor that mediates export of HIV structural mRNAs from the nucleus to cytoplasm. If such a mechanism for control of HIV gene expression does exist, it is envisioned that this factor also plays a role in the normal posttranscriptional events that govern cellular gene expression. Thus, HIV may have usurped a normal host control mechanism and added a further level of complexity.

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Expanded HIV-1 Cellular Tropism by Phenotypic Mixing with Murine Endogenous Retroviruses

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In view of the current interest in in vivo murine models for acquired immunodeficiency syndrome (AIDS), the interaction between human immunodeficiency virus type 1 (HIV-1) and endogenous murine leukemia virus (MuLV)-related retroviruses was investigated with a human leukemic T cell line (PF-382_x) that acquired xenotropic MuLV (X-MuLV) after in vivo passage in immunosuppressed mice. Despite similar levels of membrane CD4 expression and HIV-1¹²⁵I-labeled gp120 binding, a dramatic acceleration in the time course of HIV-1 infection was observed in PF-382_x compared to its X-MuLV-negative counterpart (PF-382). Moreover, PF-382 cells coinfected by X-MuLV and HIV-1 generated a progeny of phenotypically mixed viral particles, enabling HIV-1 to productively infect a panel of CD4⁻ human cells, including B lymphoid cells and purified normal peripheral blood CD4⁻/CD8⁺ T lymphocytes. Mixed viral phenotypes were also produced by human CD4⁺ T cells coinfected with an amphotropic MuLV-related retrovirus (A-MuLV) and HIV-1. These data show that endogenous MuLV acquired by human cells transplanted into mice can significantly interact with HIV-1, thereby inducing important alterations of HIV-1 biological properties.

T IS WELL ESTABLISHED THAT HIV IS the causative agent of AIDS and related disorders (1). The need for analytical approaches to the study of AIDS pathogenesis and for large-scale testing of new drugs and vaccines has prompted the search for experimental animal models of HIV infection. On the basis of phylogenetic related-

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ness, nonhuman primates represent the most suitable candidates. Persistent infection with HIV-1 has been achieved in chimpanzees (Pan troglodytes) (2) and gibbon apes (Hylobates lar) (3), whereas rhesus macaques (Macaca mulatta) have been infected with HIV-2 (4), a retrovirus remarkably similar to some pathogenic strains of the simian immunodeficiency virus group (5). However, no definitive evidence of immunodeficiency or other disease has been observed to date in these animals (6). Recently, small animal-model systems for HIV-1 infection and, possibly, pathogenicity have been proposed. These included rabbits (Oryctolagus cuniculi) (7), transgenic mice (Mus musculus) (8) and mice homozygous for the severe combined immune deficiency (SCID) mutation that had been transplanted with human hematopoietic cells (SCID-hu) (9).

A critical prerequisite for animal models of infectious diseases is that the experimental agent be substantially unaltered after passage through the novel host. One concern is that significant interactions may occur between experimentally inoculated viruses and endogenous retroviruses. Such retroviruses have been documented in several species (10) and particularly well characterized in mice, where endogenous murine leukemia virus (MuLV)-related type C viruses exhibit a variety of host tropisms (11). Interaction between endogenous retroviruses and other viral agents can determine phenotypic alterations, such as pseudotyping and phenotypic mixing (12), or even genotypic recombination, resulting in permanently modified viral progenies (13). Endogenous retroviruses may also directly interact with xenogeneic cells transplanted into mice, as demonstrated by the efficient infection of human cells by X-MuLV both in vitro and in vivo (14). Infection by X-MuLV, in turn, may alter the immunological properties of xenogeneic cells and, possibly, contribute to their tumorigenic ability in vivo (15).

Six human hematopoietic tumors were transplanted into splenectomized, irradiated, and anti-asialo-GM1-treated nude mice (SIA-*nu/nu*), and produced localized tumors of human cell origin that were able to grow in vitro as continuous cell lines (*16*). All these cells were found to have persistently acquired in vivo a type C retrovirus charac-

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Table 1. Infection by X-MuLV of human hematopoietic cells transplanted into immunodeficient mice. Human tumor cells (5×10^{6}) were injected subcutaneously into SIA-*nu/nu* mice; localized tumors (at the inoculation site) became macroscopically visible in 10 to 15 days (*16*). We extensively minced the tumors excized from the animals and made cell suspensions that were then cultured in complete medium after repeated washings. All cell types explanted from mice were able to grow indefinitely in vitro as immortalized cell lines with different kinetics of replication (but in all the cell lines the doubling time was < 72 hours). The criteria used to detect and characterize X-MuLV in all the cases were morphological observation by electron microscopy, presence of Mn²⁺-dependent reverse transcriptase enzymatic activity, immunological recognition by specific antisera (see legend to Fig. 2), and DNA hybridization with a specific molecular probe (*28*). The xenotropism was assessed by the ability of cell-free virus stocks to productively infect human or mink cells but not mouse cells (that is, NIH 3T3 fibroblasts).

Cell type	Lineage origin	Growth in vitro		X-MuLV in
		Before SIA-nu/nu	After SIA-nu/nu	tumors from SIA-nu/nu
ST4	T cell	_	+	+
GI1	T cell	-	+	+
PF-382	T cell	+	+	+
K562	Myeloid/erythroid	+	+	+
U937	Myeloid/monocytic	+	+	+
Jurkat	T cell	+	+	+

terized as X-MuLV (Table 1). One of these cell types, which was able to grow in vitro both before (PF-382) and after (PF-382_x) in vivo passage, was chosen as a suitable model to study the interaction between HIV-1 and endogenous X-MuLV. PF-382_x cells displayed identical karyotypic markers, major histocompatibility complex (MHC) haplotype, and T cell-associated markers as the original transplanted PF-382 cells (17). To further assess whether the in vivo passage had selected subclones of PF-382 particularly susceptible to HIV-1, we studied the expression of the CD4 antigen (the HIV-1 receptor) and the binding of ¹²⁵Ilabeled HIV-1 gp120 glycoproteins before and after transplantation into mice. PF-382 and PF-382_x displayed an identical pattern of CD4 expression, as detected by fluorocytometry (Fig. 1A). Consistently, similar levels of HIV-1 gp120 binding were detected in the two cell lines (Fig. 1B). From Scatchard analysis, the calculated dissociation constant (K_d) values were $1.85 \times 10^{-9}M$ for PF-382 and $1.04 \times 10^{-9}M$ for PF-382_x. The numbers of receptor sites were 2930 per cell for PF-382 and 1854 per cell for PF-382_x. These results suggest that, at least at the receptor level, the susceptibility of PF-382 to HIV-1 was not substantially modified by the passage in vivo.

The cell lines PF-382 and PF-382_x were exposed to equal infectious titers of HIV-1 under carefully controlled conditions. Although both cell types were susceptible to productive and cytopathic infection, a dramatic acceleration of HIV-1 antigen expression was seen in PF-382_x compared to its X-MuLV-negative counterpart. By day 9 after infection, radioimmunoprecipitation assay

Fig. 1. Surface membrane CD4 antigen expression (A) and binding of 12 ⁵I-labeled HIV-1 gp120 glycoprotein (B) in PF-382 (X-MuLV⁻) and PF-382_x (X-MuLV⁺). The presence of CD4 (solid line) was monitored with the fluorescein-labeled monoclonal antibody Leu3a. An irrelevant antibody (G1) was used as a negative control (dotted line). For the HIV-1-gp120 binding studies, purified envelope glycoproteins from HIV-1 [strain 451 (30)] were labeled with ¹²⁵I as previously described (31) and incubated for 1 hour at 37°C with serially diluted cells. The amount of bound radioactivity was measured by a gamma counter.



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(RIPA) of [³⁵S]cysteine-labeled PF-382 cells revealed only faint bands corresponding to the HIV-1 envelope glycoproteins gp160 and gp120. By contrast, the complete antigenic profiles of HIV-1 and X-MuLV could be readily detected in PF-382_x (Fig. 2A). Indirect immunofluorescence assay (IFA), performed at 3-day intervals, conclusively demonstrated an accelerated time course of HIV-1 expression in PF-382_x (Fig. 2B). Consistent with this finding, a significant acceleration of the HIV-1-induced cytopathic effect (that is, syncytia formation) was also observed (Fig. 2C). Analogous results were obtained with PF-382 cells persistently infected in vitro with the X-MuLV produced by $PF-382_x$ cells (18), thus indicating that the effect was directly related to the presence of X-MuLV and confirming that PF-382_x does not represent a highly HIV-1-susceptible clone of the original cell line. In addition, exposure to X-MuLV for 1 hour before HIV-1 infection resulted in an accelerated HIV-1 expression in normal human peripheral blood T lymphocytes (18).

In an attempt to elucidate the possible

Fig. 2. Time course of HIV-1 infection in PF-382 and PF-382_x cells. (A) Radioimmunoprecipitation assay (RIPA) of [³⁵S]cysteine-labeled cells for the detection of specific proteins of X-MuLV (a) and HIV-1 (b) at day 9 after infection. The assay was as described (32). The antisera used were rabbit antibody to MuLV envelope (lanes 1a); rabbit antibody to MuLV gag (lanes 2a); negative rabbit serum (lanes 3a); human HIV⁺ patient serum (lanes 1b); cocktail of monoclonal antibodies to HIV-1 p24 gag and antibody to HIV-1 gp120 env (lanes 2b); and negative human serum (lanes 3b). Cells (10×10^6) from either cell line collected during the exponential growth phase were washed twice with complete culture medium (RPMI 1640 supplemented with 10% fetal bovine serum) at 37°C, pelmechanism for the accelerated time course of HIV-1 infection in X-MuLV-infected cells, we tested for transactivation of the HIV-1 long terminal repeat (LTR) in PF-382 and PF-382_x using a recombinant plasmid construct containing the complete LTR of HIV-1 linked to the CAT reporter gene (19). In both cell lines, equally low to undetectable levels of transactivation of the HIV-1 LTR were observed (20).

We subsequently investigated the hostrange characteristics of the viral progeny released by X-MuLV/HIV-1-coinfected PF-382 cells. In these cells, both HIV-1 and X-MuLV had normal profiles of their major structural proteins (Fig. 2A), including the envelope glycoproteins that are critically involved in determining the viral tropism. Nonetheless, when the viral progeny generated by coinfected PF-382 cells were used for infection, HIV-1 was able to penetrate into and be expressed by a panel of previously nonsusceptible CD4⁻ human cells: HeLa (neoplastic epithelial cells), 501-T (diploid lung fibroblasts), A204 (neoplastic muscle cells), Raji (neoplastic B cells), and extensively purified CD8⁺/CD4⁻ normal periph-



letted, and each pellet was resuspended in approximately 10^7 median tissue culture infectious dose (TCID₅₀) of HIV-1 (strain HTLV-III_B, grown in the Molt-3 human T cell line) in 2 ml. After 1 hour at 37°C, the cells were washed twice and cultured in 10 ml of complete medium. The gp160 and gp85 represent the envelope protein precursors for HIV-1 and X-MuLV, respectively. The gp120 and gp69/70 represent the corresponding *env* proteins. The p53 and p65 represent the *gag* protein precursors in HIV-1 and X-MuLV, respectively. The high molecular weight band (~180 kD) immunoprecipitated on lane 1 does not correspond to any known MuLV envelope gene product and should be considered a nonspecific reactivity of this particular rabbit serum. (**B**) Indirect IFA detection of HIV-1 p24 antigen expression in acetone-fixed PF-382 (x) and PF-382_x cells (O) at various times after infection. A monoclonal antibody to HIV-1 p24 *gag* was used. (**C**) Morphological features of PF-382 (a) and PF-382_x (b) cells 9 days after infection with HIV-1. A cytopathic effect (that is, syncytia formation) was detectable in X-MuLV/HIV-1–infected cells, whereas cells infected with HIV-1 alone showed comparable levels of cytopathicity only at days 15 to 17 after infection.

eral blood T lymphocytes (Table 2). In addition, a CD4⁺ T cell line (CEM₅₀) could be productively infected with HIV-1 even in the presence of inhibitory concentrations of the monoclonal antibody OKT4a (Table 2). Along with HIV-1, X-MuLV was also simultaneously expressed in all the cell types tested. Analogous results were obtained with the supernatants of CEM₅₀ cells coinfected by HIV-1 and a well-characterized amphotropic MuLV-related retrovirus (A-MuLV) (21) (Table 2). These latter supernatants also allowed HIV-1 penetration and expression in cells of nonhuman origin, such as the mink lung cell line NBL-7 (American Type Culture Collection) (18).

To identify the possible emergence of genotypic recombinants between HIV-1 and X-MuLV, we studied the restriction map of the HIV-1 proviral DNA that was integrated in A204 cells after infection with phenotypically mixed HIV-1, as well as that passaged seven times in CEM₅₀ cells in association with X-MuLV. After digestion with three different restriction endonucleases, the map of HIV-1 was found unaltered by Southern (DNA) blot analysis in both cell types (18) using the BH10 probe. If ever HIV-1/X-MuLV recombinants were generated within coinfected cells, their presence was quantitatively negligible, failing to reach the threshold for detection by Southern blot analysis. In addition, complete profiles of both X-MuLV and HIV-1-specific proteins were detected by RIPA in coinfected A204 cells and were identical to those observed in singly infected controls or in coinfected PF-382 cells (18).

The observed broadening of the cellular tropism of HIV-1 may be ascribed to the generation of pseudotypes containing the HIV-1 genome coated by the envelope of X-MuLV or, more likely, of phenotypically mixed viral particles simultaneously exhibiting X-MuLV and HIV-1 glycoproteins. In an attempt to serially passage phenotypically mixed HIV-1 in CD4⁻ cells, uninfected A204 cells were incubated with the supernatant fluid of coinfected A204 cells (collected 5 days after exposure to the viral progeny of HIV-1-infected PF-382_x cells). Only X-MuLV, but not HIV-1, could be passaged beyond the first cycle of infection (18). This observation suggests that the process of de novo mixed phenotype formation in coinfected CD4⁻ cells was very inefficient. By contrast, both X-MuLV and HIV-1 were passaged up to seven times by cell-free transmission in CEM₅₀ cells, which are susceptible to both viruses (18).

The presence of endogenous MuLV-related retroviruses has been shown in virtually all laboratory strains of mice, including those currently being investigated as poten-

tial animal model systems for AIDS (that is, SCID-hu/HIV-1 mice and HIV-1 transgenic mice) (11). Since human cells of different lineage efficiently acquire X-MuLV after transplantation into immunosuppressed mice, it is presumable that in the SCID-hu/HIV-1 model, where positive engraftment of human hematopoietic cells occurs, HIV-1 could be directly interacting with X-MuLV. As we show here, this could result in an anomalously accelerated time course of HIV-1 infection. In addition, coinfection of individual human cells by X-MuLV and HIV-1 could generate phenotypically mixed virions, causing the CD4independent spread of HIV-1 to previously nonsusceptible target cells.

Phenotypic mixing is a well-documented phenomenon and has been shown between HIV-1 and vesicular stomatitis virus or herpes simplex virus (23). In our system, we have observed that diverse human CD4cells, including B cells and CD8⁺/CD4⁻ T lymphocytes, can be infected by HIV-1 after phenotypic mixing with X-MuLV. This finding is particularly pertinent to the SCID-hu/HIV-1 model, in which two characteristic hematological features almost invariably present in HIV-1-infected humans are notably absent, that is, B-lymphocytic activation with polyclonal hypergammaglobulinemia (24) [in SCID-hu/HIV-1 a dramatic decrease of immunoglobulin (Ig) production was instead reported] and CD8⁺ T-lymphocytic activation with increased proportions of circulating CD8⁺ T cells expressing class II MHC antigens (25).

The action of X-MuLV-related viruses might also help to explain the almost complete absence of graft-versus-host disease in SCID-hu after transplant of immunologically competent human immune cells into a T cell-deficient host (9). In fact, preliminary experiments in our laboratories indicate that normal human CD8⁺ T lymphocytes exposed for 1 hour to X-MuLV lose their ability to respond to alloantigenic stimulation in mixed leukocyte culture. These results are consistent with previous reports on the immunosuppressive activity of the envelope p15E transmembrane protein that is present in both MuLV and feline leukemia virus (26). In this respect, the effects of X-MuLV on human cells may represent a problem for the use of SCID mice for studies on the physiology and development of human hematopoiesis.

Another murine model proposed for use in the investigation of the pathogenesis of AIDS is the germ line insertion of the complete HIV-1 genome as a transgene (8). Here, the virus-to-virus interaction would become even more complex, since all somatic and germinal cells in these animals would simultaneously coharbor HIV-1 and endogenous murine retroviruses of both xenotropic and ecotropic host range. In addition to mice, the presence of endogenous retroviruses was suggested also in rabbits (27), another species recently proposed as a possible in vivo model for AIDS. Therefore, the potential interaction of HIV-1 with endoge-

Table 2. HIV-1 infection of CD4⁻ human cells by phenotypic mixing with X-MuLV. The presence of HIV-1 was evaluated by indirect IFA with monoclonal antibodies to HIV-1 gag p24 and env gp120, by molecular hybridization with specific DNA probes and by RIPA. The virus used for infection was produced by the cell lines $PF-382_x$ or CEM_{50}/A -MuLV infected with HIV-1 (strain HTLV-VIII_B). The supernatant fluids were collected 7 days after infection (when >80% of the cells resulted productively coinfected). As a control, the supernatant fluid of CEM₅₀ cells infected with HIV-1 alone, containing equal levels of Mg²⁺-dependent reverse transcriptase, was used. All cell types used, with the exception of CEM₅₀, were negative for the CD4 antigen by direct IFA and fluorocytometry. We purified normal CD8⁺ T lymphocytes from the peripheral blood of adult volunteers by repeated negative selection [first on quiescent mononuclear cells and then 7 days after in vitro activation with purified phytohemagglutin (1 µg/ml) by a rosetting technique (29). A cocktail of monoclonal antiobodies [OKT4a and OKT4 (Ortho Diagnostics), LeuM3, LeuI6, and LeuI9 (Becton Dickinson)], and goat antibody to mouse IgG-coated magnetic beads (Dynal) were used for selection. Experiments testing for infection inhibition by OKT4a on CEM₅₀ cells were performed as follows: cells were treated with OKT4a at the concentration of 4 μ g/ml for 30 min at 4°C, then the virus (at the approximate multiplicity of infection of 1) was added for 1 hour at 37°C and subsequently washed repeatedly with cold phosphate-buffered saline. The antibody was maintained at the same initial concentration for the entire culture period.

Target	Lineage	Infection by HIV-1 7 days after exposure to:		
cells	origin	HIV-1	HIV-1/ X-MuLV	HIV-1/ A-MuLV
HeLa	Neoplastic epithelium	_	+	+
501-T	Diploid lung fibroblasts	_	+	+
Raji	Neoplastic B lymphoid	_	+	+
A204	Neoplastic skeletal muscle	_	+	+
CD8 ⁺ /PHA	Normal PB CD8 ⁺ T lymphoid	_	+	+
CEM ₅₀ /OKT4a	CD4 ⁺ neoplastic T lympoid + OKT4A	-	+	+

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nous type C viruses should be considered in these animals.

Our observations should prompt a critical evaluation of the experimental data obtained from some animal models for HIV-1 and of their suitability for use in the study of new antiviral therapeutic or prophylactic approaches. A note of caution concerning the biosafety measures is also suggested, in consideration of the potential in vivo generation of more pathogenic phenotypic or genotypic variants of HIV-1.

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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 (KATP) channels are present in brain cells and play a role in neurosecretion at nerve terminals. KATP 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. KATP channel inhibition leads to activation of γ -aminobutyric acid (GABA) release, whereas KATP 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.

TP-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. KATP 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 KATP channels.

The central nervous system is a rich source of sulfonylurea receptors (9). These receptors are present in high concentrations in

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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).



K_{ATP} channels are permeable to both K⁺ and Rb⁺. ⁸⁶Rb⁺ efflux (13) has been useful for identifying KATP 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. [³H]GABAevoked release by (A) extracellular potassium [K⁺]_o, concentration (B) glucose, and (C) gliquidone. Horizontal bars represent the period of stimulation by effectors. (A) Control in medium (\bullet), activation by 15 mM K⁺ (\diamond). 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 mM. (B) Control in medium (•), activation by 20 mM glucose dose-re-(∇). Inset, sponse curve for glucose activation (at 10 min). (C) Control (●), activa-

tion by 100 nM gliquidone (∇). Inset, dose-response curve for gliquidone activation (at 5 min). (**D**) Release of $[^{3}H]GABA$ was evoked by increasing concentrations of gliquidone (\diamondsuit) (EC₅₀, 3 nM), LH35 (\triangle) (EC₅₀, 50 nM), glipizide (\bigcirc) (EC₅₀, 80 nM), LH32 (\checkmark) (EC₅₀, 500 nM), glipenclamide (\blacktriangle) (EC₅₀, 27.5 μ M), glisoxepide (\blacklozenge) (EC₅₀, 27.5 μ M), and tolbutamide (\bigtriangledown) (EC₅₀, 300 μ M). Data points represent the means of four experiments.

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