contain evidence of anticellular antibodies. Results presented here therefore demonstrate that SIV envelope antigens alone are sufficient to elicit protective immunity in macaques against a low-dose intravenous challenge by the homologous virus. Similarly, envelope glycoproteins of HIV-1 have also been shown to elicit protective immunity in the chimpanzee model (*24*).

A successful vaccine must be able to protect against multiple viral isolates. The immunized macaques described here generated antibodies that neutralized not only the homologous strain of SIV but also an uncloned stock of SIV*mac*251, which is approximately 9% divergent from SIV*mne* in the *env* region (*25*). It remains to be shown whether recombinant subunit vaccines could protect against heterologous virus challenge, as has been demonstrated for whole inactivated SIV vaccines (*26*).

A combination immunization regimen similar to this model is now under evaluation in humans as candidate AIDS vaccines (*27*). Although protection in the SIV system does not necessarily predict efficacy in humans against HIV-1 infection, our findings do argue for further testings of this combination immunization approach to define the limits and the correlates for protection in animal models and, ultimately, to determine efficacy in humans.

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20. The in vitro titer of the challenge stock of SIV*mne* E11S was 1×10^6 to 9×10^6 tissue culture infectious dose (TCID) per milliliter on AA2 CL 1 cells. Rhesus macaques (three or four animals per group) inoculated intravenously with 1 ml of this stock virus diluted 10^3- or 10^4-fold were 100% infected, whereas one of four animals inoculated with 10^5 dilution was infected and none at 10^6 or higher (R. E. Benveniste and G. Eddy, in preparation). Of three rhesus macaques inoculated with E11S at 10^3 dilution (*10*), all developed CD4 cell depletion and one died at 116 weeks after infection. E11S (at 10^1 to 10^3 dilution) has also been inoculated intravenously into six *Macaca nemestrina* at the Washington Primate Center. All animals died between 19 and 153 weeks after inoculation; four had CD4 cell depletion and the other two died with thrombocytopenia before significant CD4 cell depletion was evident (L. Kuller, W. M. Morton, and R. E. Benveniste, in preparation).
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Modulation of Activity of the Promoter of the Human *MDR*1 Gene by Ras and p53

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Drug resistance in human cancer is associated with overexpression of the multidrug resistance (*MDR*1) gene, which confers cross-resistance to hydrophobic natural product cytotoxic drugs. Expression of the *MDR*1 gene can occur de novo in human cancers in the absence of drug treatment. The promoter of the human *MDR*1 gene was shown to be a target for the c-Ha-Ras-1 oncogene and the p53 tumor suppressor gene products, both of which are associated with tumor progression. The stimulatory effect of c-Ha-Ras-1 was not specific for the *MDR*1 promoter alone, whereas a mutant p53 specifically stimulated the *MDR*1 promoter and wild-type p53 exerted specific repression. These results imply that the *MDR*1 gene could be activated during tumor progression associated with mutations in Ras and p53.

DRUG RESISTANCE IS A MAJOR OBstacle to the successful chemotherapy of human malignancies. The expression of the human *MDR*1 gene, which encodes an energy-dependent efflux pump, is responsible for the resistance of tumor cells to various hydrophobic cytotoxic drugs (*1*). *MDR*1 RNA and its product, P-glycoprotein (also called the multidrug transporter), are found at substantial levels in normal colon, small intestine, kidney, liver, and adrenal gland (*2*) as well as in capillaries of the brain and testis (*3*). The localization of P-glycoprotein on the apical surface of transporting epithelia and endothelia suggests that it may have a normal physiological role in transporting cytotoxic compounds or metabolites. However, the endogenous substrate or substrates of the multidrug transporter remain unknown. In rat liver, expression of *mdr* RNA is modulated in response to toxic insults such as carcinogens and *mdr* RNA is increased in regenerating liver after partial hepatectomy and in mouse uterus by progesterone (*4*). *MDR*1 gene expression is regulated by heat-shock, arsenite, and cadmium in a human kidney cell line and also by chemotherapeutic agents in rodent cells (*5*).

Expression of the *MDR*1 gene occurs commonly in human cancers derived from normal tissues that express the multidrug transporter, such as carcinomas of the colon, liver, kidney, pancreas, and adrenal gland, and may contribute to the broad spectrum drug resistance of these cancers (*6*). In addition, *MDR*1 RNA levels in acute leukemias, neuroblastoma, pheochromocytoma, ovarian cancers, and breast cancers are also increased following relapse after chemotherapy, suggesting that *MDR*1 expression may be selected in tumor populations exposed to

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chemotherapy. However, substantial *MDR*1 gene expression may also occur in some cancers not derived from *MDR*1-expressing normal tissues and not previously exposed to drugs such as acute nonlymphocytic leukemias and chronic myelogenous leukemia in blast crisis (*6*). These results raise the possibility that expression of the *MDR*1 gene is regulated in some way by the same genes that drive tumor progression.

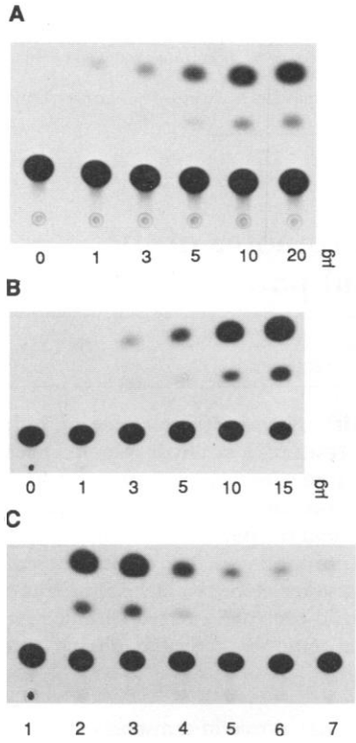

Fig. 1. Dose-dependent stimulation of MDRCAT expression by p53 mutant (**A**) and Ras (**B**). The human *MDR*1 gene promoter construct, MDRCAT, contains 1.8 kb of genomic sequences upstream from the initiation codon (ATG) of the human *MDR*1 gene, cloned into pSV00CAT directly in front of the CAT gene (*26*). Approximately 5×10^5 NIH 3T3 cells were plated in a 100-mm culture dish and then cotransfected with 5 μg of MDRCAT and various amounts of Ras or the mutant p53 plasmids by the calcium phosphate–DNA precipitation transfection procedure (*27*). Most transfections contained 20 μg of total DNA, with sonicated salmon sperm DNA (Pharmacia) as carrier. The CAT activity was determined approximately 40 hours after transfection with equivalent amounts of protein extracts. The CAT activity was quantified by scintillation counting of the percentage of acetylated ^{14}C-labeled chloramphenicol (*25*). (**C**) Inhibition of mutant p53 stimulated MDRCAT expression in NIH 3T3 cells by wild-type p53. Cells were either transfected with 5 μg of MDRCAT, lane 1; cotransfected with 5 μg of MDRCAT and 10 μg of mutant p53 (p53MUT), lane 2; or transfected with 5 μg of MDRCAT, 10 μg of p53MUT and various amounts of wild-type p53 as follows: lane 3, 0.3 μg; lane 4, 1 μg; lane 5, 3 μg; lane 6, 5 μg; and lane 7, 10 μg.

The *ras* oncogene and the p53 tumor suppressor gene have been implicated in the malignant progression of cancers such as carcinoma of the colon, neuroblastoma, and chronic myelogenous leukemia in blast crisis (*7*). Because Ras proteins affect gene expression (*8*), and recent observations suggest that p53 protein (*9*) might function directly as a transcriptional regulator (*10*), we speculated that the emergence of multidrug resistance might be closely associated with tumor progression. The *MDR*1 gene could be turned on as a result of activation of *ras* and inactivation of the p53 tumor suppressor gene, which occur through genetic lesions during tumorigenesis. We examined this hypothesis directly by cotransfecting *MDR*1 promoter–chloramphenicol acetyltransferase (CAT) constructions with expression vectors for c-HA-*RAS*-1 and wild-type and mutant p53.

Our human *MDR*1 gene promoter–CAT construction, MDRCAT, consisted of 1.8 kb of genomic sequences linked to the bacterial CAT reporter gene. When we transiently cotransfected NIH 3T3 cells with equal amounts of an expression vector encoding either a mutant human p53 cDNA (Fig. 1A) harboring a substitution from arginine to histidine at amino acid 175 (*11*) or the c-Ha-Ras-1 cDNA (Fig. 1B) driven by the SV40 promoter (*12*), the CAT activities were increased 7- to 180-fold in a dose-dependent manner. In comparison, controls cotransfected either with a plasmid carrying the neomycin resistance gene driven by the same promoter or with an enhancerless, promoterless CAT vector (pSV0CAT) gave no stimulation (*13*). The stimulation of MDRCAT expression by mutant p53 was reversed by cotransfecting with the wild-type p53 in a dose-dependent manner (Fig. 1C, lanes 3 to 7), with 50% inhibition of CAT activity at 1 μg of cotransfected wild-type p53 DNA (Fig. 1C). Mutant p53 and Ras had additive effects on MDRCAT expression, and Ras-stimulated CAT expression was not inhibited by wild-type p53 in NIH 3T3 cells (*13*), suggesting that Ras and p53 acted independently on the MDR gene promoter.

Transformation of rat liver epithelial cells with v-Ha-*ras* or v-*raf* increases drug resistance and *mdr* gene expression (*14*). To ascertain whether the effect of Ras was related to transformation, we transiently transfected NIH 3T3 and Kirsten-*ras*–transformed NIH 3T3 cells (KNIH) with MDRCAT. Basal CAT activity was low in NIH 3T3 cells, and approximately sixfold higher in KNIH cells, with both cell types showing dose dependence on MDRCAT DNA (Fig. 2A). The β-galactosidase cDNA served as an internal control for variations in

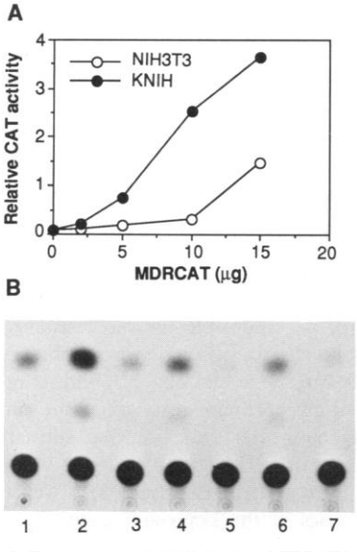

Fig. 2. Expression of MDRCAT in NIH 3T3 and KNIH cells. (**A**) NIH 3T3 and KNIH cells were transfected with various amounts of MDRCAT plasmids. A β-galactosidase gene driven by the Rous sarcoma virus long terminal repeat (LTR) (RSV-β gal) (1 μg) was cotransfected as an internal control to monitor transfection efficiency. (**B**) KNIH cells were cotransfected with 5 μg of MDRCAT and 5 μg of various effector plasmids (as described in the legend to Fig. 1). Lane 1, MDRCAT; lane 2, Ras + MDRCAT; lane 3, Ras + p53 + MDRCAT; lane 4, Ras + p53MUT + MDRCAT; lane 5, p53 + MDRCAT; lane 6, p53MUT + MDRCAT; and lane 7, p53 + p53MUT + MDRCAT.

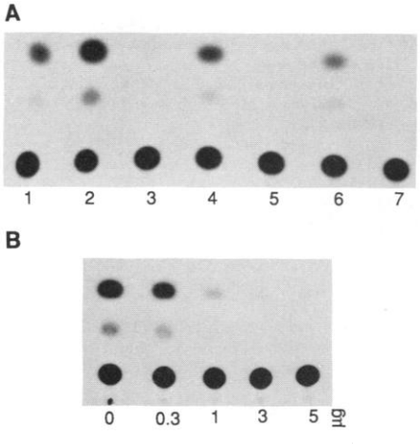

Fig. 3. Effect of Ras, p53, and p53 mutant on the expression of MDRCAT in SW13 cells. (**A**) 1.2×10^6 SW13 cells were plated and then cotransfected with MDRCAT (5 μg) and various effector plasmids (5 μg) (as in Fig. 1). Lane 1, MDRCAT; lane 2, Ras + MDRCAT; lane 3, Ras + p53 + MDRCAT; lane 4, Ras + p53MUT + MDRCAT; lane 5, p53 + MDRCAT; lane 6, p53MUT + MDRCAT; lane 7, p53 + p53MUT + MDRCAT. (**B**) Inhibition of MDRCAT expression in SW13 cells. Cells were cotransfected with MDRCAT (5 μg) and various amounts of wild-type p53 expression plasmid as indicated, and CAT assays were performed.

Table 1. Effect of Ras, wild-type, and mutant p53 on the expression of gene promoter–CAT fusions in NIH 3T3 cells. Cotransfections of the effector plasmids (5 μg) and the promoter-CAT constructs (2 μg) were conducted as in Fig. 1. Relative CAT activity was obtained from the ratio of the percentage of conversion of ^{14}C-labeled chloramphenicol to acetylated chloramphenicol in the presence of effector plasmids, over the control transfected with pSV2NEO (NEO).

Construct*	Relative CAT activity			
	NEO	Ras	p53	p53MUT
ACTINCAT	1	18	1.3	0.6
EGFRCAT	1	0.9	3.2	1.3
MDRCAT	1	6.8	0.9	7.4
RASCAT	1	4.1	1.2	1.2
RSVCAT	1	7.2	0.3	0.3
pSV2CAT	1	4.9	0.1	0.7

*ACTINCAT, chicken β-actin promoter–CAT fusion construct (*21*); EGFRCAT, human epidermal growth factor receptor gene promoter–CAT fusion (*22*); MDRCAT, human multidrug resistance gene promoter–CAT fusion; RASCAT, human Harvey ras proto-oncogene promoter–CAT fusion (*23*); RSVCAT, Rous sarcoma virus LTR-CAT fusion (*24*); pSV2CAT, SV40 gene promoter-CAT fusion (*25*).

transfection efficiency, and the CAT activities were normalized to the β-galactosidase activities, which were comparable for NIH 3T3 and KNIH cells. These results indicate that the transformed KNIH cells were more permissive for the expression of MDRCAT. When KNIH cells were cotransfected with MDRCAT and the other plasmids described above, CAT activity was further increased in the presence of Ras, and its stimulation was not reversed by cotransfecting either with the wild-type or with the mutant p53 plasmids (Fig. 2B). Cotransfection with wild-type p53 but not with the mutant strongly repressed the high basal MDRCAT expression. Because similar results were obtained with NIH 3T3 and transformed KNIH cells, Ras-mediated stimulation of MDRCAT expression does not appear to be related to the transforming effect of Ras.

To examine the inhibitory effect of wild-type p53 in a cell line that allows high basal expression of MDRCAT, we use the human adrenocortical carcinoma cell line, SW13. The wild-type p53 gene reduced MDRCAT expression by approximately 60-fold, whereas mutant p53 and Ras had no pronounced effects on the already high activity of the reporter plasmid (Fig. 3A). The repression by p53 was dose-dependent, with a maximal inhibition of CAT expression at approximately 3 μg of the test plasmid (Fig. 3B).

To determine the specificity of the effects of Ras and p53, we examined the influence of c-Ha-Ras-1, wild-type p53, and mutant p53 on the promoter activities of other genes. In addition to the human *MDR*1 gene promoter, cotransfection of c-Ha-Ras-1 enhanced activity of promoter-CAT constructions with promoters for chicken β-actin, c-Ha-Ras, Rous sarcoma virus (RSV), and SV40 (Table 1). Of the promoters tested, only the epidermal growth factor (EGF) receptor promoter showed no stimulation by Ras. Whereas Ras seemed to be a rather promiscuous stimulator of many different promoters, the effects of p53 and its mutant were more specific. Wild-type p53 had no significant effects on the β-actin, *ras*, and EGF receptor promoters but reduced RSV and SV40-promoter–driven CAT expression. Mutant p53 enhanced only MDRCAT expression, and somewhat reduced CAT expression driven by RSV and SV40 promoters. These experiments have been repeated three times, and the results obtained are qualitatively consistent. No significant stimulation of EGF receptor promoter by wild-type p53 was observed in subsequent experiments.

We have also cotransfected NIH 3T3 and KNIH cells with MDRCAT and various other oncogenes such as c-*fos*, c-*jun*, or c-*myc* to further verify the specificity of the stimulation caused by Ras and p53 (*13*). Stability of these DNAs was indicated by the preservation of transforming activity. However, no significant stimulation in the MDR promoter activity was observed in these experiments, suggesting that the MDR promoter activity could only be modulated by activation or inactivation of certain oncogenes and tumor suppressor genes.

To assess the state of the p53 proteins in NIH 3T3, KNIH, and SW13 cells, we immunoprecipitated extracts from these cells with monoclonal antibody PAb 421, selected for reactivity to wild-type and mutant p53 (*15*), or with monoclonal antibody PAb 240, which is specific for certain mutant forms of p53 (*16*). Although a specific band of p53 was detected in NIH 3T3, KNIH, and SW13 cells with antibody 421, mutant p53 was detected with antibody 240 only in SW13 cells and not in NIH 3T3 or KNIH cells (Fig. 4). The human osteogenic sarcoma Saos-2 cell, which lacks the functional p53 (*17*), was included in this analysis as a negative control; and the human epidermoid carcinoma A431 and the hepatocellular carcinoma PLC/PRF/5 cells, which are known to harbor mutant p53 (*18*), were included as positive controls. These results indicate that NIH 3T3 and KNIH cells contain mainly wild-type p53 and SW13 cells contain the mutant protein.

Our results show that the *MDR*1 gene promoter is a potential target for p53 and Ras. The mutant p53 stimulated MDRCAT expression in NIH 3T3 cells, and the observed enhancement was completely abolished when the cells were cotransfected with wild-type p53. In contrast to recent findings that wild-type p53 but not mutant p53 may be involved in activation of transcription (*10*), our data suggest that wild-type p53 may be capable of repressing gene transcription either directly or indirectly. We observed stimulation of MDRCAT expression by mutant p53 in NIH 3T3 cells, which are normally nonpermissive for MDRCAT expression, and inhibition of MDRCAT expression by wild-type p53 in SW13 cells, which are permissive for MDRCAT expression. One simple hypothesis to explain this result is that wild-type p53 is present in NIH 3T3 cells and reduces expression of MDRCAT in these cells. Introduction of mutant p53 may reduce the negative effect of wild-type p53 by its dominant negative effect (*9*). In SW13 cells by this model, the predominant form of p53 would be a mutant form, allowing high basal level expression of MDRCAT. Introduction of mutant p53 would have no further effect, whereas wild-type p53 should strongly inhibit MDRCAT expression. The presence of mutant p53 detected by the conformation-dependent antibody PAb 240 in SW13 cells, and the presence of wild-type but not mu-

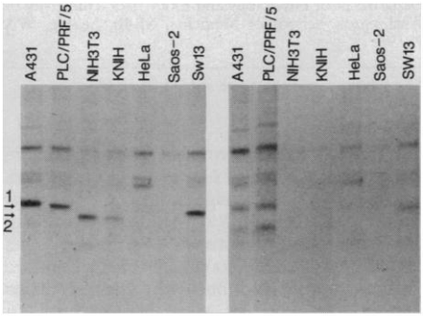

Fig. 4. Immunoprecipitation analysis of p53. Approximately 4 × 10^6 cells were plated overnight and then labeled with 100 to 200 μCi/ml of ^{35}S-methionine (ICN; Tran ^{35}S-Label) for 2 hours. Cells were lysed and extracts were obtained for immunoprecipitation. Approximately 12 × 10^6 trichloroacetic acid–precipitable counts were immunoprecipitated with monoclonal antibodies PAb 421, which recognize both wild-type and mutant p53 (*15*), or PAb 240, which recognizes only mutant p53 (*16*). The immunoprecipitated proteins were then analyzed on 10% SDS–polyacrylamide gel electrophoresis (PAGE) and fluorographed. Positions of the human p53 (hp53) and murine p53 (mp53) are indicated by the numbers 1 and 2, respectively. The human osteosarcoma Saos-2 cell, which lacks p53 (*17*), the human cervical carcinoma HeLa cell, which has a low concentration of p53 mRNA but no detectable p53 protein (*18*), the epidermoid carcinoma A431, and the hepatocellular carcinoma PLC/PRF/5 cells (*28*), which contain mutant p53, are included as controls in the experiments.

tant p53 in NIH 3T3 cells detected with antibody PAb 421 but not PAb 240 (Fig. 4), lends support to this hypothesis.

An alternative model to explain our observations is that both wild-type and mutant p53 can activate or repress transcription in a promoter- and cell-type–specific manner. In one sense, our observations are reminiscent of the effect of the viral erb A oncogenic protein, which normally inhibits gene activation in animal cells but unexpectedly functions as a hormonally regulated transcriptional activator in *Saccharomyces cerevisiae* (*19*). The mutant p53 used in this study could be a transcriptional activator, whereas wild-type p53 could be a repressor in NIH 3T3 cells. In KNIH and SW13 cells, mutant p53 does not act as an activator but also does not inhibit activity as does wild-type p53, suggesting that it has lost its ability to act as a repressor of *MDR*1 transcriptional activation in these cell types.

It is now generally agreed that genetic alterations in oncogenes combined with functional loss of tumor suppressor genes may account for the onset and development of a number of human cancers, and that Ras and p53 mutations are clearly involved in malignant transformation (*20*). Our results, therefore, may provide the basis for a model describing the molecular events that are involved in the regulation of *MDR*1 gene expression during the progression of human cancers.

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GnRH-Induced Ca^{2+} Oscillations and Rhythmic Hyperpolarizations of Pituitary Gonadotropes

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Secretion of gonadotropic hormones from pituitary gonadotropes in response to gonadotropin-releasing hormone (GnRH) is essential for regulation of reproductive potential. Gonadotropes from male rats exhibited an unusual form of cellular excitation that resulted from periodic membrane hyperpolarization. GnRH induced an oscillatory release of intracellular Ca^{2+} via a guanosine triphosphate (GTP) binding protein–coupled phosphoinositide pathway and hyperpolarized the gonadotrope periodically by opening apamin-sensitive Ca^{2+}-activated K^+ (SK) channels. Each hyperpolarization was terminated by firing of a few action potentials that may result from removal of inactivation from voltage-gated Na^+ and Ca^{2+} channels.

PITUITARY GONADOTROPES SECRETE luteinizing hormone and follicle-stimulating hormone in response to pulses of the peptide GnRH in the pituitary portal circulation. The biochemical responses of gonadodotropes to GnRH include an increase in phosphoinositide (PI) turnover, a rise in the con- centration of intracellular free Ca^{2+}, and activation of protein kinase C (PKC) (*1–5*). But the electrophysiological events underlying the stimulation-secretion coupling are less well known because of the difficulty of identifying gonadotropes in the heterogeneous population of pituitary cells. In sheep, GnRH has been reported to induce no change of gonadotrope membrane potential or resistance (*6*). In rat, the initial phase of GnRH-induced secretion of luteinizing hormone is reported not to require extracellular Ca^{2+}, but the later phase (after 2 min) does and is diminished by dihydropyridine Ca^{2+} channel blockers (*7*). In a gonadotrope cell line, GnRH potentiates voltage-gated Ca^{2+} currents (*4*), possibly by a PKC-dependent mechanism (*8*). Using gigaseal recording techniques (*9*) on identified gonadotropes of the male rat (*10–12*), we now show that GnRH induces rhythmic release of Ca^{2+} from the inositol 1,4,5-trisphosphate (IP_3)–sensitive store and that Ca^{2+} entry via voltage-gated Ca^{2+} channels may occur during action poten-

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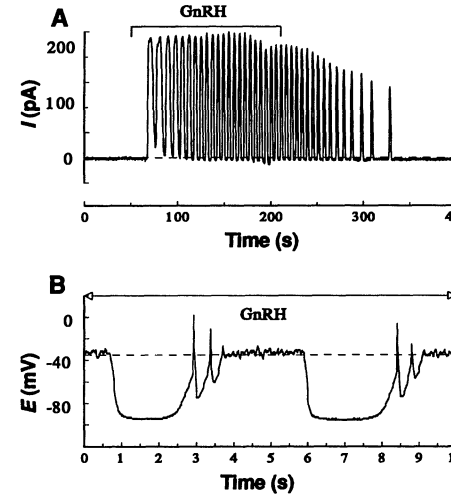

Fig. 1. Oscillations of membrane current and membrane voltage induced by GnRH. (**A**) Ionic currents (*I*) of a gonadotrope voltage clamped at −50 mV as GnRH solution (10 nM) was perfused into the bath during the 160-s period marked with a bar. In other cells exposed to GnRH continuously for 15 min, the rhythmic oscillations of outward current continued without decrement. (**B**) Membrane potential (*E*) changes of a gonadotrope continuously exposed to GnRH (100 nM) in the bath. Application started 3 min before the beginning of the trace. The dashed line denotes the resting potential (−35 mV) of this cell before GnRH was applied.