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Antagonistic Adrenergic-Muscarinic Regulation of M Current in Smooth Muscle Cells

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The β-adrenergic agonist isoproterenol and analogs of adenosine 3′,5′-monophosphate (cAMP) induced a potassium current, M current, in freshly dissociated gastric smooth muscle cells. Muscarinic agonists suppress this current, apparently by acting at a locus downstream from regulation of cAMP levels by adenylate cyclase and phosphodiesterase. Thus, M current can be induced by an agent and regulated in antagonistic fashion by β-adrenergic and muscarinic systems.

THE MODULATION OF IONIC CHANnels by neurotransmitters occurs via a variety of second messengers, including cyclic nucleotides, guanosine triphosphate (GTP)–binding proteins, calcium ions, and metabolites of phosphatidylinositol and arachidonic acid (*1–3*). Dual regulation of the same ionic channel by transmitters exerting antagonistic effects has been documented for Ca^{2+} channels in heart (*4–6*), potassium channels in *Xenopus* ovarian follicles (*7*), and K^+ channels (S channels) in invertebrate neurons (*3, 8*).

The M current is a transmitter-regulated, voltage-sensitive K^+ current suppressed by acetylcholine acting on muscarinic receptors, which was first described in sympathetic neurons (*9*) and since has been observed in a variety of cells, including smooth muscle (*10*). A number of neuropeptides [substance P, luteinizing hormone–releasing hormone (LHRH) (*11, 12*), and bradykinin (*2*)] also suppress M current. But M current has not appeared to be susceptible to dual regulation because only its suppression, and not its induction by transmitters, has been demonstrated. We report here that M current can be induced by the β-adrenergic agent isoproterenol in isolated smooth muscle cells, an induction that can be mimicked by forskolin or phosphodiesterase-resistant analogs of adenosine 3′,5′-monophosphate (cAMP). Hence, this current is regulated in antagonistic fashion by muscarinic and β-adrenergic systems. Some of these results have appeared in preliminary form (*13*).

The effects of isoproterenol on ionic currents were studied in smooth muscle cells freshly dissociated from the stomach of the toad *Bufo marinus* (*14*). Voltage-clamp was accomplished with either conventional microelectrodes (*15*) or with patch electrodes in the whole-cell configuration (*16*). Records from a cell held at a depolarized level and periodically hyperpolarized are shown in Fig. 1. Isoproterenol caused the slow development of outward current at −15 mV, which appears as an upward displacement in the holding current. In response to hyperpolarization of the membrane, the initial rapid current jump (or "ohmic jump"), which is directly proportional to the membrane conductance, increased upon isoproterenol application (compare Fig. 1, a and b). Furthermore, after isoproterenol, the ohmic jump upon hyperpolarization from −15 to −65 mV was followed by a marked slow inward current relaxation, which represents the turning off of the isoproterenol-induced outward current at −65 mV (*10*). This voltage-dependent turnoff of the underlying conductance accounts for the absence of isoproterenol effects at more negative potentials.

The outward current activated by isoproterenol persisted for several minutes but was quickly abolished by acetylcholine (ACh). Suppression of the outward current was accompanied by a decrease in the ohmic jump, indicative of a conductance decrease, and elimination of the inward current relaxations, reflecting the abolition of voltage-dependent current (compare Fig. 1, c and d). The decrease in conductance and absence of current relaxations demonstrate that ACh acts by suppressing outward current and not by activating an opposing inward current. [ACh applied to the cell before any exposure to isoproterenol caused a smaller decrease in conductance, indicating the presence of some M current prior to isoproterenol application, which we designate "endogenous"

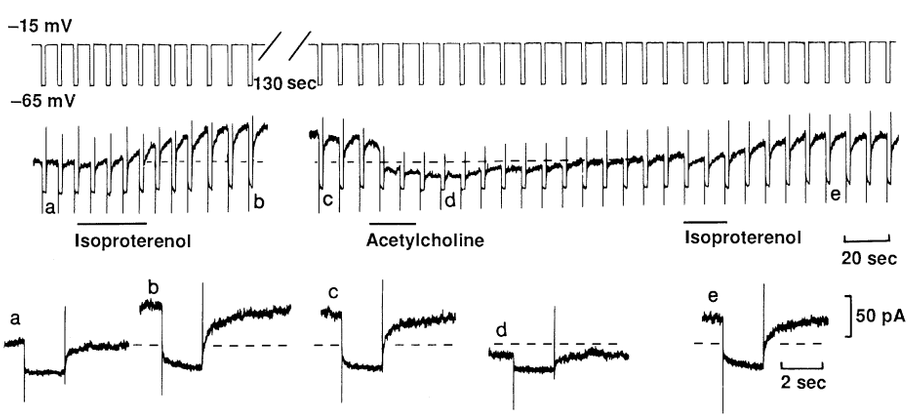

Fig. 1. Isoproterenol activates outward current in smooth muscle cell. (Upper trace) Membrane potential; holding potential, −15 mV, with periodic hyperpolarizations to −65 mV. (Lower trace) Current, with selected responses to hyperpolarizing commands shown on an expanded time scale. Isoproterenol caused slow development of outward current (upwards) above the control dashed line. The induced outward current exhibited voltage sensitivity as indicated by the current relaxations (compare expanded trace a with traces b and c). (Inward current relaxations at the onset of the hyperpolarizing pulses represent K^+ current turning off. Outward current relaxations, at the offset of the pulses, represent the slow activation of K^+ current but with some contamination by voltage-activated Ca^{2+} current.) The magnitude of endogenous M current is indicated by the shift of the current below the control level at −15 mV after ACh application (trace d). Another measure of the endogenous M current is provided by comparing the ohmic current jumps at the onset of the hyperpolarizing pulse in traces a and d; the difference is due to endogenous M current. At −65 mV there is little difference in steady-state current (compare traces a and d), because M current is largely deactivated at this potential. Inward current relaxation due to the endogenous M current is not readily apparent in trace a, because the magnitude of this current is relatively small and its relaxations are faster than those of induced M current. A conventional microelectrode was used in this experiment. The results illustrated are representative of responses from 44 cells.

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M current and which is considerably larger in many cells (*10*).] After recovery from ACh, a second application of isoproterenol again caused development of outward current accompanied by an increase in conductance and restoration of voltage-dependent current relaxations (Fig. 1e) (*17*). Thus, isoproterenol activates a conductance that is voltage-sensitive, that remains steadily activated at depolarized potentials to cause an outward current, and that turns off upon hyperpolarization. Furthermore, this conductance was suppressed by ACh.

Two observations indicate that the isoproterenol-activated conductance is selective for K^+. First, cells were bathed in solution containing an elevated extracellular K^+ concentration of 30 m*M*, where the K^+ reversal potential (E_K) was −37 mV (KCl exchanged for NaCl, leaving Cl^- unchanged). With the same procedure as used in Fig. 1, isoproterenol induced outward current at −15 mV (a potential more positive than E_K) and inward current at −65 mV (a potential more negative than E_K), both of which were suppressed by ACh (eight cells). Second, we investigated the ionic basis of current relaxations induced by isoproterenol (and suppressed by ACh) by stepping through a range of potentials. In 3 m*M* extracellular K^+, isoproterenol-induced current relaxations reversed direction at −80 ± 3.3 mV (SD, n = 10), close to the reversal level expected for K^+ and to the reversal level observed for endogenous M current (*18*).

A quantitative description of the K^+ conductance activated by isoproterenol was determined from steady-state current-voltage (*I-V*) plots (Fig. 2). Outwardly rectifying current was induced by isoproterenol and subsequently suppressed by the selective agonist muscarine. The conductance activated by isoproterenol (g_M, see below) was determined as the difference between control and steady-state isoproterenol current levels divided by the driving force for K^+ (Fig. 2C). The g_M increased, in a sigmoidal fashion, with depolarization, an increase that was fitted by a Boltzmann relation:

$$g_M = g_{M(max)}(1 + \exp[(V_{0.5} - V_m)/A])^{-1}$$

Nonlinear, least-squares fit gave the following values: maximum conductance ($g_{M(max)}$) = 1314 pS; voltage at half-activation ($V_{0.5}$) = −53 mV; and slope factor (A) = 8 mV, values similar to those obtained in three other cells. Because the instantaneous *I-V* relation was linear over the relevant range of potentials, g_M is proportional to the probability that a K^+ channel activated by isoproterenol is open at each potential in the steady state.

The current elicited by isoproterenol, therefore, fulfills the criteria for M current. That is, the induced current is outward current carried by K^+ ions, is suppressed by muscarine or ACh, remains steadily activated at positive potentials, turns off with hyperpolarization, and exhibits slow relaxations in response to voltage jumps. In addition, the slope factor in the Boltzmann relation is similar to that reported previously for M current (*9, 10*).

β-Adrenergic stimulation of many smooth muscles, including these gastric cells, causes relaxation, which is associated in some instances with hyperpolarization (*19, 20*) and is thought to be mediated by the cAMP second messenger system (*21*). We examined the role of cAMP in activation of M current by application of the membrane permeant analogs, 8-bromo–cAMP and dibutyryl cAMP. Like isoproterenol, cAMP analogs induced voltage-dependent outward current (eight cells with 8-bromo–cAMP and two cells with dibutyryl cAMP) (Fig. 3). The current relaxations reversed direction at the same level as isoproterenol-induced current (−80 ± 3.6 mV, n = 4). The outward current induced by 8-bromo–cAMP was suppressed by muscarine, as indicated by the conductance decrease and disappearance of slow current relaxations (Fig. 3A) (*22*). The *I-V* relation resulting from application of 8-bromo–cAMP (Fig. 3B) resembled that shown for isoproterenol above. Forskolin (10 μ*M*), an activator of adenylate cyclase, also elicited M current in two cells.

The fact that K^+ current induced by cAMP analogs can be suppressed by muscarine provides evidence that ACh exerts its antagonistic effect at a point "downstream" from adenylate cyclase in the β-adrenergic cascade. Furthermore, two lines of evidence argue against the possibility that ACh stimulates a cyclic nucleotide phosphodiesterase (*5*). First, 8-bromo–cAMP is highly resistant to phosphodiesterase (*23*). Second, after suppression by muscarine, the current induced by the 8-bromo–cAMP did recover (Fig. 3A) (*24*).

Our results demonstrate an effect of muscarinic agonists on isoproterenol- and cAMP-induced M current. In addition, many cells exhibit substantial endogenous M current (*10*), and in these cells isoproterenol caused development of additional outward current and increased the size of current relaxations. Both endogenous and isoproterenol-enhanced M current were suppressed by ACh. Significantly, relaxations of endogenous M current upon hyperpolarizing jumps were faster than those of isoproterenol-induced M current. Thus, isoproterenol may act by altering the kinetics of existing M channels, by activating a subpopulation of M channels that are distinguishable from those responsible for endogenous M current by their slower deactivation kinetics, or both (*25*). At present we cannot distinguish among these possibilities.

Thus, M current can be induced by an agonist, the β-adrenergic agent isoproterenol. The evidence presented here for the involvement of a cAMP-dependent mechanism is supported by the previous demonstration that cAMP levels in these cells rise rapidly following β-adrenergic stimulation (*21*). Furthermore, the M current is regulated in antagonistic fashion by the muscarinic

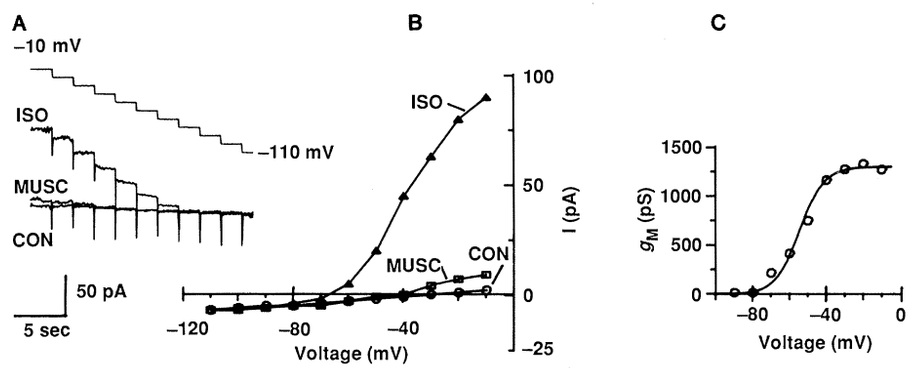

Fig. 2. Steady-state current-voltage (*I-V*) relations reveal the outward current induced by isoproterenol (ISO). (**A**) The membrane potential was progressively commanded negative in 10-mV steps (upper trace), with current shown below. We first measured the control (CON) *I-V*, then applied isoproterenol, causing development of outward current at potentials positive to −80 mV. Subsequent application of muscarine (MUSC) (100 μ*M* in the application pipette) largely suppressed the outward current; thus, the *I-V* overlaps control. The small difference between control and muscarine *I-V*s may represent a small muscarine-insensitive component but is within the range of variability we encountered. (**B**) Steady-state values are plotted for the response illustrated in (A). The difference between the isoproterenol and control *I-V* curves represents the current induced by isoproterenol. (**C**) The conductance activated by isoproterenol was determined as this current divided by driving force, using a reversal level of −80 mV (*18*). The curve is drawn according to a Boltzmann relation (see text). Standard whole-cell recording methods were used for these records and those in Fig. 3 (*16*).

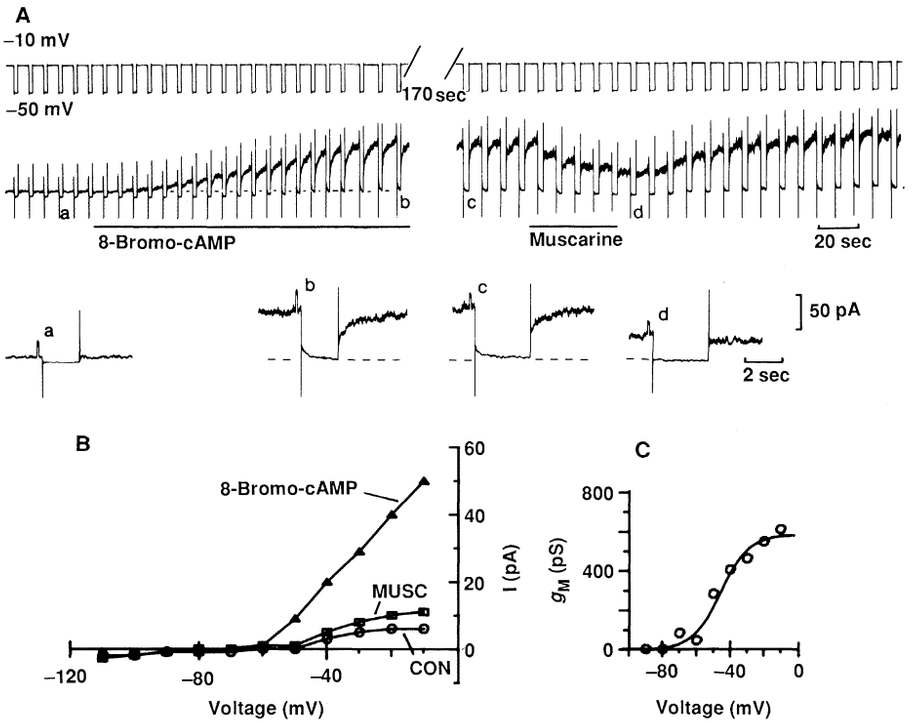

Fig. 3. Analog to cAMP induces M current. (**A**) 8-Bromo–cAMP (5 m*M* in the application pipette) caused outward current (middle trace) that exhibited voltage-dependent current relaxations. Dashed line represents control current level at −10 mV. Muscarine (50 μ*M* in the application pipette) suppressed most of the outward current. The conductance decrease is evident from the smaller ohmic current jump seen upon hyperpolarization and the suppression of current relaxations (compare traces c and d). Recovery from muscarine is seen at right. Note that the cell is somewhat "leakier" after several minutes of recording, as indicated by the larger ohmic jump. Calibration pulses (20 pA) preceded each expanded current trace. (**B** and **C**) *I-V* relation and conductance activated by 8-bromo–cAMP were determined in another cell. *I-V* curves were obtained for control, then 8-bromo–cAMP, and then muscarine. Fitted parameters for the Boltzmann relation are $g_{M(max)}$ = 594 pS, $V_{0.5}$ = −46 mV, and A = 9.5 mV. *I-V* curve in the presence of muscarine has been shifted 5 pA positive to account for a change in the leak of the cell.

and β-adrenergic systems (*26*). The mechanism of the antagonism, however, appears to be fundamentally different from that found in cardiac cells, where antagonistic muscarinic–β-adrenergic effects on ionic channels have been best studied. In cardiac cells, the muscarinic system appears to antagonize the increase in Ca^{2+} current caused by β-adrenergic agents by acting at the level of the GTP-binding proteins controlling adenylate cyclase (*6*) or perhaps at the level of phosphodiesterase (*5*). In either event the locus of the muscarinic antagonism is "upstream" of the cAMP-dependent protein kinase itself. In the smooth muscle cells that we used, however, muscarinic antagonism of M current induced by the β-adrenergic system appears to be exerted at a point downstream from control of cAMP levels by adenylate cyclase and phosphodiesterase, that is, at or beyond the cAMP-dependent kinase itself, and possibly at the level of the channels responsible for the M current. Thus, the results here point to a novel mechanism for dual antagonistic regulation of ion channels by muscarinic and β-adrenergic systems, one that allows for the interesting possibility of selective muscarinic antagonism of only some of the β-adrenergic effects.

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15. Single electrode voltage-clamp recording was with conventional microelectrodes (3*M* KCl, 30 to 50 megohms) and an Axoclamp 2 switching amplifier. Switching rate and capacitive neutralization were adjusted so that voltage measured at the headstage decayed more than 90% after each current injection cycle (cycling rate, 1.5 to 3.0 kHz). Current and voltage were recorded with a modified digital audio processor and a videocassette recorder. Bath perfusion occurred at a rate of 1 to 2 ml per minute, with a solution consisting of 94 m*M* NaCl, 3 m*M* KCl, 5 m*M* Hepes-NaOH, 20 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 5 m*M* tetraethylammonium chloride (TEA), and 11 m*M* glucose at *p*H 7.8. Test substances were applied by pneumatic pressure ejection from micropipettes of 1- to 2-μm tip size. Agonist concentrations (50 μ*M* in the application pipette) represent upper limits at the cell surface. Control application of bathing solution had no effect on currents. Acetylcholine (Kodak) and isoproterenol (Sigma) were made up as aqueous stock solutions, kept frozen, and diluted on the day of the experiment, causing no detectable change in *p*H of the solutions. Oxidation of isoproterenol was minimized by storing solutions in sealed syringes, on ice, in darkness, and by using each pipette for no more than 45 minutes. Experiments were performed at room temperature (20° to 25°C).
16. Whole-cell recording methods, with a conventional patch-clamp amplifier, were used [O. P. Hamill, A. Marty, E. Neher, B. Sakmann, F. J. Sigworth, *Pfluegers Arch.* **391**, 85 (1981)]. Patch electrodes (resistances 3 to 10 megohms) contained 130 m*M* K^+, 110 m*M* Cl^-, 10 m*M* Hepes-KOH, 1 m*M* EGTA, 1 m*M* $MgCl_2$, 1 m*M* adenosine triphosphate (ATP), and 1 m*M* GTP, *p*H 7.2. In some cases, 5 m*M* EGTA and 2.8 m*M* $CaCl_2$ were used, giving an estimated free $[Ca^{2+}]$ of ≃100 n*M*. Similar responses were less consistently observed with no ATP, GTP, or Mg^{2+} in pipettes. As compared to microelectrode recordings with the switching amplifier, recordings with patch pipettes yielded higher input resistances and quieter traces, the latter being most evident at more negative potentials where M current is largely inactivated (compare Figs. 1 and 3).
17. Close inspection reveals a small inward current during isoproterenol application associated with a conductance decrease. Such a response was only occasionally seen, was of short latency and duration, and was seen most often after extended recording from a cell. It reflects suppression of K^+ current, since it reversed direction near E_K. This "leak" current differs from M current discussed in this report, having no obvious time or voltage dependence.
18. Membrane potentials given are those at the patch pipette and have not been corrected for junction potentials (−3 to −5 mV should be added to the values). The true transmembrane potential may be somewhat more negative as a result of a Donnan potential [A. Marty and E. Neher, in *Single-Channel Recording*, B. Sakmann and E. Neher, Eds. (Plenum, New York, 1983), pp. 107–122]. This might account for reversal of M current at approximately −80 mV, compared to a calculated reversal of −95 mV which was also the value found when recording with microelectrodes (*10*).
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22. Adenosine 3′,5′-monophosphate was not reported to have any effect on M current in sympathetic ganglion neurons (*9*), possibly reflecting differences in cell systems.

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24. These observations are most simply explained by involvement of an independent pathway at a point distal to adenylate cyclase and phosphodiesterase, but it is possible to postulate more complex mechanisms involving cellular compartments or threshold phenomena.
25. Isoproterenol might increase the macroscopic M current by increasing the number of M channels available to be opened, increasing the probability of existing channels being in the open state, or both. Assuming a simple two-state model of channel gating, where α is the opening and β the closing rate constant (*9*), the time constant of channel deactivation is given by $\tau = 1/(\alpha + \beta)$. Values for τ increase with isoproterenol, indicating a decrease of $(\alpha + \beta)$. Because we are dealing with an increase in macroscopic current, we might assume that α is not decreasing, so β must be decreasing. That is, the slower kinetics of the isoproterenol current might reflect a decrease in the closing rate constant for M channels. Besides increasing time-averaged current through the single channel, this could shift the half-activation potential more negative, which is consistent with our results, since $V_{0.5}$ is more negative than expected, given the junction potential and reversal potential seen in whole-cell recording (*18*).
26. The β-adrenergic effects described here seem insufficient to account completely for the inhibitory actions of catecholamines on smooth muscle, because these effects can be counteracted by the excitatory neurotransmitter ACh. This leaves open the possibility of other cellular responses to β-adrenergic agonists.
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Transmission of HIV in Belle Glade, Florida: Lessons for Other Communities in the United States

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The high cumulative incidence of AIDS and the large percentage of AIDS patients with no identified risks in Belle Glade, Florida, were evaluated through case interviews and neighborhood-based seroepidemiologic studies. It was found that of 93 AIDS patients reported between July 1982 and 1 August 1987, 34 could be directly linked to at least one other AIDS patient or to a person with AIDS-related complex by sexual contact, sharing of needles during intravenous drug abuse (or both), or perinatal exposure; of 877 randomly selected adults, 28 had antibodies to HIV; no person over age 60 and none of 138 children aged 2 to 10 years had antibodies to HIV; no clustering of infected persons within households occurred, except in sex partners; and HIV-seropositive adults were more likely than HIV-seronegative adults to be from Haiti, have a lower income, report sex with intravenous drug abusers, and have a history of previous treatment for sexually transmitted diseases. The presence of antibodies to five arboviruses prevalent in South Florida or the Caribbean did not correlate significantly with HIV infection. The high cumulative rate of AIDS in Belle Glade appears to be the result of HIV transmission through sexual contact and intravenous drug abuse; the evidence does not suggest transmission of HIV through insects.

BELLE GLADE, FLORIDA, IS AN AGRICULTURAL community of approximately 16,500 persons. It is located southeast of Lake Okeechobee and 45 miles west of West Palm Beach. Each year, thousands of migrant farm workers, primarily American blacks, British West Indians, Haitians, and Hispanics, enter Belle Glade and surrounding areas during sugarcane and vegetable harvesting months (*1*). From July 1982 through 1 August 1987, 93 persons meeting the surveillance case definition for acquired immunodeficiency syndrome (AIDS) were reported from Belle Glade, for an estimated AIDS cumulative incidence of 564 per 100,000. Seven (8%) of these 93 persons had no identified risk factors associated with human immunodeficiency virus (HIV) infection, compared with 3% of nationally reported AIDS cases (*2*).

The high cumulative rate of AIDS cases and relatively large proportion of persons with no identified risk factors resulted in concern that environmental factors, perhaps including mosquitoes, were contributing to HIV transmission in Belle Glade. The Florida Department of Health and Rehabilitative Services (HRS), the Palm Beach County Health Unit, and the Centers for Disease Control (CDC) initiated a series of studies to determine the prevalence of HIV infection in Belle Glade residents and to define more precisely the risk factors for HIV transmission in this community (*3*).

AIDS patients were interviewed in May 1985 and in May and August 1986 by health professionals affiliated with HRS or CDC. Persons were asked about known risk factors for HIV transmission, including a history of contact with other AIDS patients or other persons at risk for HIV infection. If the patients had died before they could be interviewed, family members or known sexual contacts who provided informed consent were interviewed. Parents of children with AIDS were asked about risk factors for HIV infection and about the child's medical history.

To document the prevalence of HIV infection and modes of HIV transmission in Belle Glade, three seroepidemiologic studies were conducted between February and October 1986. Several groups were enrolled.

1) *Randomly selected participants.* A comprehensive list of household addresses was obtained for a neighborhood-based survey, and a random list was generated to recruit participants from about 500 of these on a door-to-door basis. Housing units were grouped into 12 locally defined neighborhoods (LDNs) conforming to 1980 census criteria (*4*). Sampling was weighted so that approximately 70% of households were preferentially selected from the LDNs with the largest number of reported AIDS patients. Persons 18 years of age or older who provided written informed consent and children aged 2 to 10 years whose parents or guardians provided consent were interviewed with the standardized questionnaire, asked for a blood sample, and examined for signs of HIV infection.

2) *Self-selected participants.* Persons 18 years or older (and their children if aged 2 to 10 years) who had not been selected to participate in the neighborhood-based survey but requested testing for HIV antibodies were also enrolled in the study.

3) *Clinic participants.* Because the number of participants with HIV infection was expected to be low in the neighborhood-based survey, attendees at the Glades Community Health Center in Belle Glade who were being evaluated for the possibility of HIV-related illness were invited to participate in the study. Since the self-selected participants

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