pholipids by the action of PLA_2. During our investigation of the role of phospholipids in activating $I_{K\cdot AA}$, we found that 10 μM phosphatidylserine (PS) or phosphatidylcholine (PC) activated a second K^+ channel when applied to the intracellular surface of the patch. This channel was K^+-selective, as judged by a −60 mV per decade change in slope in the plot of intracellular K^+ concentration versus reversal potential. The limiting single-channel conductance was 68 pS in the outward direction and 44 pS in the inward direction with a mean open time of ~1 ms. In cell-attached patches, 10 to 50 μM PC or PS did not cause channel activation when added to the bath or to the pipette. Lysophosphatidylcholine, a product of the action of PLA_2 on PC, activated this channel in one patch out of six. Arachidonic acid and its metabolites mentioned above (HPETEs, HETEs, and leukotrienes) had no effect on channel activation. It is unlikely that protein kinase C was involved in channel activation because phorbol esters at 10 μM [phorbol myristate acetate (PMA) and 1-oleoyl-2-acetylglycerol (OAG)] ($n \geq 183$) did not open either of these K+ channels.

The K^+ channels found in this study may play an important role in protecting against cell damage caused by ischemia, which is known to elevate intracellular levels of certain fatty acids, including arachidonic acid (*9*). Ischemia or hypoxia can reduce the duration of the action potential and thereby cause early repolarization of cardiac cells (*10*). Opening of the K^+ channels reported here would cause rapid hyperpolarization of the cell and limit additional entry of Ca^{2+} via voltage-sensitive Ca^{2+} channels as well as minimize energy consumption by conserving ATP. A decrease in intracellular *p*H together with the opening of the K^+ channels by arachidonic acid would contribute to more rapid repolarization of the cells by increasing the K^+ channel activity, further protecting the cells from ischemic damage. We cannot yet generalize our results to adult human cardiac cells, so the importance of these channels in ischemia remains speculative. Alternatively, these K^+ channels may be part of newly discovered second messenger pathways involving lipophilic compounds, as suggested by other reports of arachidonic acid metabolites in gating channels (*1, 2*). The role of these compounds as part of a physiological second messenger system is supported by the fact that the existence of these K^+ channels may not be limited to heart cells. Arachidonic acid (5 to 40 μM) and certain fatty acids activate two types of K^+ channels in gastric smooth muscle cells (*11*) when added extracellularly. We do not know whether our channels are activated directly by fatty acids such as arachidonic acid. The pathway is apparently independent of $G_{\beta\gamma}$-dependent $I_{K\cdot ACh}$ activation (*2*).

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Arachidonic Acid and Other Fatty Acids Directly Activate Potassium Channels in Smooth Muscle Cells

RICHARD W. ORDWAY, JOHN V. WALSH, JR., JOSHUA J. SINGER

Arachidonic acid, as well as fatty acids that are not substrates for cyclooxygenase and lipoxygenase enzymes, activated a specific type of potassium channel in freshly dissociated smooth muscle cells. Activation occurred in excised membrane patches in the absence of calcium and all nucleotides. Therefore signal transduction pathways that require such soluble factors, including the NADPH-dependent cytochrome P450 pathway, do not mediate the response. Thus, fatty acids directly activate potassium channels and so may constitute a class of signal molecules that regulate ion channels.

ARACHIDONIC ACID (AA) AFFECTS the behavior of biological systems in two ways. First, the liberation of this fatty acid (FA) from cell membrane phospholipid, via receptor-mediated activation of phospholipases, leads to the generation of biologically active AA metabolites (that is, products of cyclooxygenase, lipoxygenase, and cytochrome P450 metabolic pathways) (*1*). Such metabolic conversion of AA can result in the activation of K^+ channels (*2*). A second class of responses is elicited by AA itself and by other FAs. These responses do not proceed through the metabolic pathways for AA and are referred to as "direct" FA effects. The most convincing demonstration of this second class of responses is the direct activation of purified enzymes by FAs (*3–6*). FA modulation of cellular processes (*7–11*) may reflect similar direct interactions of FAs with proteins. Here we report that both AA and certain other FAs, at concentrations similar to those required for both metabolically mediated (*1, 2, 12*) and direct (*4–6, 7–10*) effects of FAs, directly activate specific K^+ channels in smooth muscle cells. Thus certain FAs, liberated by receptor-regulated lipases or by other processes, may be second messenger molecules for the regulation of ion channels. Some of these results have appeared in preliminary form (*13*).

The effects of FAs were examined in smooth muscle cells, isolated from the stomach of the toad *Bufo marinus*, by using tight-seal, whole-cell and patch recording techniques (*14*). In the whole-cell configuration, AA (10 to 40 μM) rapidly and reversibly activated an outwardly rectifying K^+ current (Fig. 1). In a typical experiment, the membrane potential was intermittently stepped to values negative and then positive to the calculated equilibrium potential for K^+ (E_K). The activated K^+ current was seen as a divergence in the current trace with inward current generated at −110 mV (negative to E_K) and outward current at −60 mV (positive to E_K) (Fig. 1A). At a constant internal (patch pipette) K^+ concentration, current-voltage (*I-V*) relations for AA-activated current were obtained at different external K^+ concentrations (Na^+ exchanged for K^+) (Fig. 1B). A plot of the reversal potentials for AA-activated current as a function of the extracellular K^+ concentration (Fig. 1C) approximated that predicted for a K^+ current.

To determine whether activation of K^+ current required metabolism of AA by cyclooxygenase or lipoxygenase, we took ad-

Department of Physiology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655.

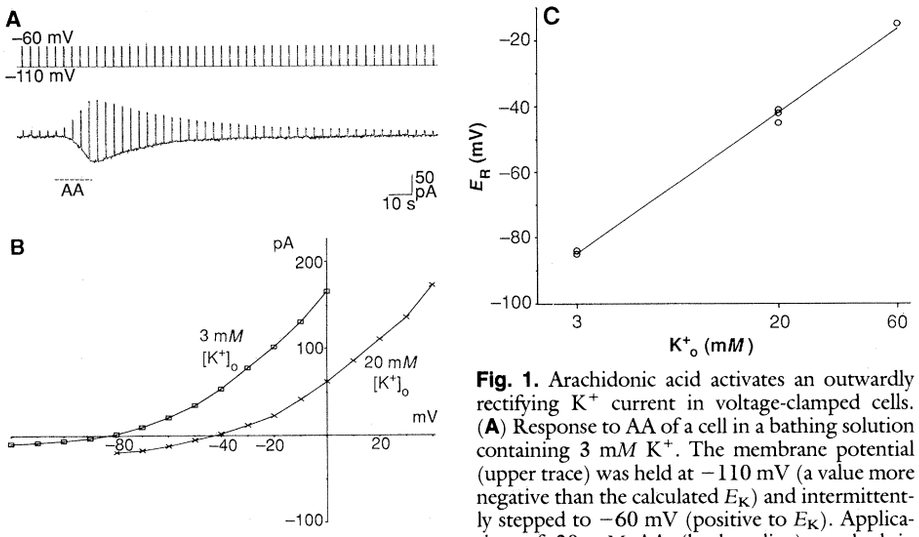

Fig. 1. Arachidonic acid activates an outwardly rectifying K^+ current in voltage-clamped cells. (**A**) Response to AA of a cell in a bathing solution containing 3 m*M* K^+. The membrane potential (upper trace) was held at −110 mV (a value more negative than the calculated E_K) and intermittently stepped to −60 mV (positive to E_K). Application of 20 μ*M* AA (broken line) resulted in generation of inward current at −110 mV and outward current at −60 mV, consistent with an increase in K^+ conductance. Bathing solution used here contained 1.8 m*M* Ca^{2+} and no EGTA (see methods). (**B**) *I-V* relations of AA-activated current in 3 and 20 m*M* external K^+ ($[K^+]_o$). The plotted currents (AA-activated currents) represent the difference between currents recorded in the presence and absence of AA during a descending staircase of voltage steps (10 mV, >1-s steps) (*30, 32*). (**C**) Reversal potentials of AA-induced current as a function of extracellular K^+ concentration. The reversal potentials at each K^+ concentration were taken as the zero current crossing in plots such as those in (B). Slope, 53 mV per 10-fold elevation in $[K^+]_o$.

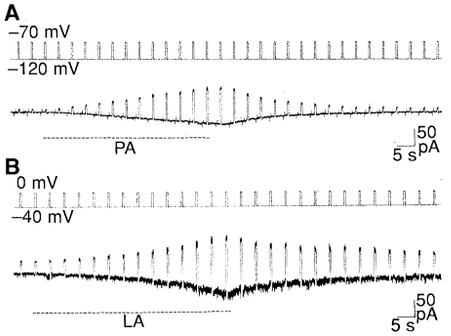

Fig. 2. Whole-cell responses to AA are mimicked by fatty acids that are not substrates for cyclooxygenase and lipoxygenase enzymes. (**A**) Response to palmitoleic acid (PA) (40 μ*M*) of a cell bathed in 3 m*M* K^+. The membrane potential (upper trace) was intermittently stepped from a holding potential of −120 mV to −70 mV (potentials negative and positive to E_K, respectively). Palmitoleic acid activated inward current at −120 mV and outward current at −70 mV (lower trace). (**B**) Response to linoelaidic acid (LA) (40 μ*M*) of a cell bathed in 60 m*M* K^+. Linoelaidic acid activated inward current at −40 mV and outward current at 0 mV, potentials respectively negative and positive to E_K. The increased noise in this current trace, as compared to other traces shown, is due to the different range of potentials employed (*33*).

vantage of the substrate specificities of these two enzymes. AA is a 20-carbon FA with cis double bonds at positions 5, 8, 11, and 14 [designated (20:4 *cis*-5,8,11,14)] and thus contains multiple *cis*-1,4 pentadiene groups (that is, methylene-interrupted cis double bonds). Although FAs that lack a 1,4 pentadiene unit with at least one cis double bond are not substrates for either cyclooxygenase or lipoxygenase enzymes (*15*), FAs lacking this feature mimicked the activation of K^+ current by AA. Responses to two such FAs, palmitoleic (16:1 cis-9) and linoelaidic (18:2 trans-9,12) acids, are shown in Fig. 2.

Potassium current was also activated by certain other FAs, including two additional FAs that are not substrates for cyclooxygenase and lipoxygenase enzymes, oleic acid (18:1 *cis*-9) (20 to 40 μ*M*) and the shorter chain saturate, myristic acid (14:0) (10 to 40 μ*M*). Linolenic acid (18:3 *cis*-9,12,15) (40 μ*M*), which is a potential substrate for oxygenases, was effective as well. Eicosatetraynoic acid (ETYA), the acetylenic analog of AA that inhibits cyclooxygenase, lipoxygenase, and cytochrome P450 enzymes (*16*), also rapidly and reversibly activated K^+ current (30 μ*M*). FAs that did not activate K^+ current under the same conditions include the more hydrophilic FAs, myristoleic (14:1 cis-9) (20 to 40 μ*M*) and caprylic (8:0) (40 to 100 μ*M*) acids. Although the longer chain saturated FA, palmitic acid (16:0) (40 μ*M*), also did not activate K^+ current, this may reflect insufficient delivery of this FA to the cell, since palmitic acid was poorly soluble in our solutions. A similar profile of FA activity has been seen in other systems (*5, 7, 9, 17*). The effectiveness of only certain FAs in these systems has been attributed to the physical properties of the different FAs in aqueous solutions and membranes, rather than to the selectivity of FA binding sites (*17, 18*). FAs activated K^+ current in the absence of nucleotides [for example, adenosine triphosphate (ATP), guanosine triphosphate (GTP), nicotinamide adenine dinucleotide phosphate (NADPH)] and Ca^{2+} (5 m*M* EGTA in both the bath and the dialyzing patch pipette solution). Virtually all cells tested exhibited FA-activated K^+ current (*19*).

The effects of FAs on excised patches were examined to determine whether well-defined K^+ channels were responsible for the FA-activated K^+ current and whether soluble cytosolic factors were required. AA (20 to 100 μ*M*) (Figs. 3A and 4) as well as FAs that are not substrates for cyclooxygenase and lipoxygenase enzymes, such as myristic acid (40 μ*M*) (Figs. 3B and 4A), activated the same type of K^+ channel in excised outside-out patches. Four additional FAs that activated whole-cell K^+ current—linoelaidic, linolenic, palmitoleic (each at 40 μ*M*), and ETYA (30 μ*M*)—were studied in outside-out patches and activated the same type of K^+ channel. The effects of FAs were also examined in both cell-attached and excised, inside-out membrane patches. AA (20 to 100 μ*M*) and myristic acid (20 μ*M*) activated the same K^+ channel in both cell-attached and inside-out patches, but onset and recovery were slower than in outside-out patches. The effectiveness of FAs in cell-attached and inside-out patches demonstrates that channel activation is not specific to FA applied to the extracellular face of the membrane and indicates that intracellular FA may be important to the regulation of this channel. FA activation of K^+ channels occurred in virtually all patches tested (*20*).

I-V relations were obtained at three different external K^+ concentrations for single channel currents activated by FAs. In 20 m*M* extracellular K^+, myristic acid (Fig. 4A) activated the same outwardly rectifying 23-pS K^+ channel activated by AA (conductance values calculated from outward currents). Linoelaidic acid (Fig. 4B) also mimicked AA activation of this channel type. In 60 m*M* K^+, the outward rectification is less marked and the channel conductance is approximately 30 pS. The unitary *I-V* relation for this channel in 3 m*M* K^+ yielded a conductance of 19 to 20 pS. Zero-current potentials shifted as predicted for a K^+ channel when the external K^+ concentration was changed.

As was the case with whole-cell responses, FA activation of K^+ channels in excised patches occurred in the absence of Ca^{2+} (5 m*M* EGTA) and nucleotides. Responses could be elicited repeatedly in excised patches for the lifetime of the patch (>1 hour). Our data indicate that Ca^{2+}, nucleotides, and other water soluble cytosolic factors are

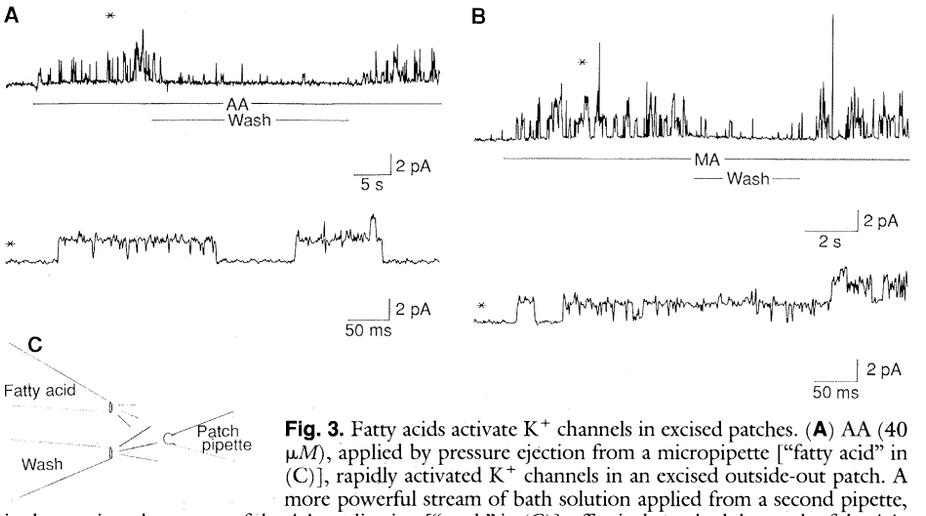

Fig. 3. Fatty acids activate K^+ channels in excised patches. (**A**) AA (40 μM), applied by pressure ejection from a micropipette ["fatty acid" in (C)], rapidly activated K^+ channels in an excised outside-out patch. A more powerful stream of bath solution applied from a second pipette, in the continued presence of the AA application ["wash" in (C)], effectively washed the patch of the AA, resulting in a marked decline in channel activity. When this second stream was turned off, the AA was again able to reach the patch, and channel activity again developed (*34*). The region of the upper trace marked with an asterisk is shown in the lower trace on an expanded time scale. The holding potential was +40 mV; $[K^+]_o$ was 3 mM. Data was filtered at 300 Hz and then sampled at 2 kHz, displayed, and plotted with customized software on a DEC PDP11 computer. Every 109th point is plotted in the top trace; every point in the bottom trace. (**B**) Myristic acid (MA) (40 μM) activated K^+ channels in an excised outside-out patch. The experimental procedure was the same as in (A) and illustrated in (C). Unlike in (A), both the fatty acid and wash solutions in this experiment contained 0.1% DMSO, which does not affect the experimental outcome. The holding potential was +50 mV; $[K^+]_o$ was 20 mM. Data was processed as in (A). Every 32nd point is plotted in the top trace; every point in the bottom trace. (**C**) The arrangement of pressure ejection pipettes in a typical experiment. Experiments were conducted as described in (A). This method allows the observation of rapid changes in FA-activated channel activity and provides controls for minor turbulence created by the pipette application technique. Routine controls for pipette application were also performed with the wash pipette at the same ejection pressure used for delivering FAs.

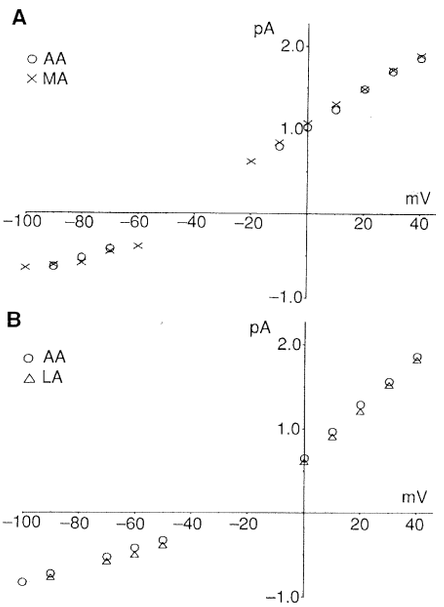

Fig. 4. *I-V* relations for FA-activated K^+ channels in excised outside-out patches. (**A**) *I-V* relations of the same type of K^+ channel activated by AA and myristic acid (MA) in 20 mM K^+ (two different patches). The conductance (measured in the linear outward current region) is 23 pS in each case. Zero-current potentials are close to the values predicted by the Nernst relation. (**B**) *I-V* relations of the same type of K^+ channel activated by AA and linoelaidic acid (LA) in the same patch (60 mM K^+). The conductance (measured in the linear outward current region) was 30 pS under these conditions.

not required for the activation of K^+ channels by FAs. Thus signal transduction mechanisms that are dependent upon such factors, including NADPH-dependent cytochrome P450–mediated FA metabolism (*1, 21*) and energy-dependent processes, do not mediate the response. These results, together with the effectiveness of FAs that are not substrates for cyclooxygenase and lipoxygenase enzymes, suggest that FAs themselves activate K^+ channels through a direct action at a protein or lipid site in the membrane.

There are precedents for the direct activation of proteins by FAs. Purified preparations of both protein kinase C (*5*) and membranous guanylate cyclase (*17*) are activated by a series of FAs similar to that described here. Additionally, AA and other FAs directly activate purified, soluble guanylate cyclase (*22, 23*). Both protein kinase C (*5*) and soluble guanylate cyclase (*23*), like K^+ channels, can also be activated by ETYA, a commonly used inhibitor of AA metabolism (*16*). (It is clear that under our experimental conditions, these enzymes cannot mediate activation of K^+ channels by FAs.)

Alternatively, channel activation may be mediated by a FA-induced alteration of the physical properties of the membrane lipid. However, a clear link between such alterations caused by FAs or other lipophilic compounds (alcohols, anesthetics, and so forth) and the effects of these compounds on ion channels and other proteins (*24*) has not been established. Cis-unsaturated fatty acids have been suggested to modulate cellular proteins by increasing the fraction of the membrane comprising fluid lipid domains (*11*). This type of effect does not explain our results, since both the saturated myristic acid and the trans-unsaturated linoelaidic acid activated K^+ channels (*7, 9, 10*).

Our results demonstrate that a number of FAs themselves, as opposed to products of the cyclooxygenase, lipoxygenase, and cytochrome P450 pathways, directly activate K^+ channels in smooth muscle cells (*25*). A similar mechanism, in lacrimal glands, has been proposed for the modulation of gap junctions (*26*).Thus, unesterified FAs associated with cell membranes and the processes that modulate these compounds physiologically may be important in the control of cell excitability. In smooth muscle cells, an increase in FAs would result in hyperpolarization and subsequent antagonism of contractile activity. Levels of FAs may be controlled by numerous processes including agonist-stimulated and basal phospholipase activity (*27*), alterations in the metabolism of unesterified FA (*27*), and fluctuations in extracellular sources of FA due to hormonal status or tissue injury (*28*). Thus the potential regulatory roles for FAs in the control of ion channels range from a lipid-derived second messenger to a signal carried via the circulation.

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14. We used whole-cell and patch recording methods (*29*) and a conventional patch clamp amplifier. Patch electrodes contained 130 m*M* K^+, 5 m*M* EGTA-KOH, 1 m*M* Mg^{2+}, 114.5 m*M* Cl^-, 10 m*M* Hepes-HCl (*p*H 7.2). In some cases less EGTA was used (1 m*M*), which did not affect the results. Cells were usually bathed in a solution containing 3 m*M* K^+, 127 m*M* Na^+, 5 m*M* EGTA-NaOH, 1 m*M* Mg^{2+}, 114.5 m*M* Cl^-, 10 m*M* Hepes-NaOH, 10 m*M* glucose (*p*H 7.8). Exceptions are noted in the figure legends. Elevation of bath K^+ concentrations was achieved by substitution of K^+ for Na^+. FA solutions were prepared by either direct dispersal of the FA oil or dispersal of concentrated FA in dimethyl sulfoxide (DMSO) into the appropriate aqueous salt solution. DMSO (0.1%) alone in bathing solution had no effect. Because of the limited solubility of FAs in physiological salt solutions, the actual concentrations in these experiments are likely to be lower than the reported values. FA solutions were applied to cells by either pressure ejection from a micropipette (*30*) or, less often, by superfusion. For the former, concentrations given are those in the application pipette. FAs (>99% pure) were obtained from Nu Check Prep with the exception of ETYA (98% pure), which was obtained from Fluka and Biomol. For all whole-cell voltage clamp recordings shown, capacitive transients were removed for clarity. Membrane potentials are given with respect to the zero current reference potential before contact of the cell by the pipette. Thus the true membrane potential is likely to be somewhat more negative, giving recorded reversal potentials that are more positive than those calculated for a channel permeable only to K^+ (*31, 32*).
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18. Consistent with this interpretation, the more hydrophilic FA, myristoleic acid, was found to weakly activate K^+ current at a concentration of 80 μ*M* (four experiments: two whole-cell recordings, two outside-out patches). This result suggests that higher concentrations of the more hydrophilic FAs result in interaction with a hydrophobic site associated with K^+ channel activation.
19. The reproducibility of whole-cell K^+ current activation by FAs is indicated by the following summary of the experimental results (expressed as the ratio of positive results to the number of cells tested): Arachidonic acid (29/30 cells); palmitoleic acid (4/4 cells); linoelaidic acid (5/5 cells); linolenic acid (3/3 cells); oleic acid (5/5 cells); myristic acid (12/12 cells); ETYA (7/7 cells). Negative results (expressed as the total number of cells tested): Myristoleic acid (20 to 40 μ*M*) (8 cells); caprylic acid (8 cells) (in 2 additional cells, caprylic acid weakly increased K^+ current after prolonged, high-pressure applications); palmitic acid (two cells).
20. The reproducibility of K^+ channel activation in membrane patches is indicated by the following summary of the experimental results [expressed as in (*19*)]: Arachidonic acid (5/6 outside-out, 11/12 cell-attached, 2/2 inside-out); myristic acid (5/5 outside-out, 4/4 cell-attached, 2/2 inside-out); linoelaidic acid (4/4 outside-out); linolenic acid (2/3 outside-out); palmitoleic acid (3/3 outside-out); ETYA (3/3 outside-out).
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25. Although we have demonstrated a direct activation of K^+ channels by FAs, in the case of AA we have not eliminated the possibility that conversion to active metabolites may also contribute to the response.
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33. The results indicate the range of responses and do not necessarily reflect the relative potencies of these FAs.
34. A second channel type, which can be seen as a smaller amplitude channel current in the upper trace of Fig. 3A, was activated by AA in some patches. Because this channel was seen infrequently, we have not yet characterized it or its activation by FAs.
35. Supported by NSF DCB-8511674 and DCB-8819750 and NIH DK-31620. We thank C. Baker, P. Tilander, and P. Beane for assistance, and M. Kirber, T. Honeyman, K. Madden, D. Melchior, and D. Small for helpful comments and suggestions.

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Transgenic Mice with I-A on Islet Cells Are Normoglycemic But Immunologically Intolerant

J. BÖHME, K. HASKINS, P. STECHA, W. VAN EWIJK, M. LEMEUR, P. GERLINGER, C. BENOIST, D. MATHIS

Insulin-dependent diabetes mellitus (IDDM) is caused by a specific loss of the insulin-producing beta cells from pancreatic Langerhans islets. It has been proposed that aberrant expression of major histocompatibility complex (MHC) class II molecules on these cells could be a triggering factor for their autoimmune destruction. This proposal was tested in transgenic mice that express allogeneic or syngeneic class II molecules on the surface of islet cells at a level comparable with that normally found on resting B lymphocytes. These animals do not develop diabetes, nor is lymphocyte infiltration of the islets observed. This immunological inactivity does not result from tolerance to the "foreign" class II molecules.

THE DESTRUCTION OF PANCREATIC beta cells in IDDM and the consequent abrogation of insulin secretion appear to have an autoimmune origin (*1*). Lymphocyte infiltration is common in islets from diabetic patients and is usual in islets from two animal models of the disease, the BioBreeding (BB) rat and the nonobese diabetic (NOD) mouse (*1–3*). In addition, the rodent diseases can be transmitted from a diabetic to a healthy individual by transferring lymphocyte populations (*4*).

As is the case with several other autoimmune diseases, IDDM seems to be associated with particular MHC alleles (*5, 6*). The reasons for this association are much in debate. Some diabetic patients express MHC class II molecules on their islet cells, as do diabetic BB rats and NOD mice (*7–9*). This observation has provoked the hypothesis that aberrant expression of MHC molecules may play a role in the autoimmune destruction of beta cells, possibly by allowing the presentation of previously ignored islet cell antigens (*5, 10*). The recent finding (*11*) that cultured beta cells can be induced to express class II molecules in vitro by lymphokine stimulation suggests an in vivo mechanism for such aberrant expression. An alternative interpretation, that the appearance of class II molecules on islet cells is secondary to the inflammatory response, rests on the timing of expression in BB rats (*8*).

The two interpretations are potentially

J. Böhme, M. LeMeur, P. Gerlinger, C. Benoist, D. Mathis, Laboratoire de Génétique Moléculaire des Eucaryotes du CNRS—Unité 184, de Biologie Moléculaire, et de Génie Génétique de l'INSERM—Institut de Chimie Biologique, Faculté de Médecine, 67085 Strasbourg Cedex, France.
K. Haskins and P. Stecha, Barbara Davis Center for Childhood Diabetes, University of Colorado Health Sciences Center, Denver, CO 80262.
W. van Ewijk, Department of Immunology, Erasmus University, Rotterdam 3000 DR, the Netherlands.