Amylin is a 37-amino acid cytotoxic constituent of amyloid deposits found in the islets of Langerhans of patients with type II diabetes. Extracellular accumulation of this peptide results in damage to insulin-producing β cell membranes and cell death. We report here that at cytotoxic concentrations, amylin forms voltage-dependent, relatively nonselective, ion-permeable channels in planar phospholipid bilayer membranes. Channel formation is dependent upon lipid membrane composition, ionic strength, and membrane potential. At 1–10 μM, cytotoxic human amylin dramatically increases the conductance of lipid bilayer membranes, while non-cytotoxic rat amylin does not. We suggest that channel formation may be the mechanism of cytotoxicity of human amylin.

Amylin is a peptide hormone co-secreted with insulin from the β cells of the islets of Langerhans in the pancreas (1–3). It is postulated that amylin has a regulatory function that opposes the action of insulin (4, 5). The pathology of non-insulin-dependent (type II) diabetes mellitus is characterized by an extracellular accumulation of fibrillar amyloid, which consists largely of amylin (6–8). The amount of amylin deposited is proportional to the insulin requirements of the patient and thus to the clinical severity of the disease (6). It has recently been reported that human amylin is toxic to islet β cells (9). Although it is not clear why amylin forms amyloid deposits, the mechanism of amylin cytotoxicity is believed to be the interaction of amyloid with β cell membranes (10–12).

In addition to humans, only a few other mammals, such as primates (3) and cats (14) are known to form amyloid deposits and develop type II diabetes. Rats are among the organisms that do not form amyloid and do not develop type II diabetes (15, 16). It has been shown in vitro that amyloidogenic human amylin is more toxic than nonamyloidogenic rat amylin (9).

Amylin has been shown to interact with phosphatidylcholine liposomes resulting in drastic changes in its secondary structure. The peptide was transformed from a combination of α-helical and β-sheet structures to a largely β-sheet structure (17). The structure of noncytotoxic rat amylin, which is mainly disordered in aqueous solution, was not affected by liposomes.

Based on the above evidence, we supposed that the mechanism of amylin toxicity might be an increase in β cell membrane permeability to ions. We therefore investigated the interaction of amylin with planar lipid bilayer membranes.

**EXPERIMENTAL PROCEDURES**

**Materials—**Human amylin (HPLC grade, >99% purity) was purchased from Bachem California, Torrance, CA, and rat amylin (HPLC grade, >98% purity) was from Bachem Biosciences, King of Prussia, PA. 1,2-Dipalmitoyl-sn-glycerol-3-phosphocholine, 1-α-phosphatidylcholine (plant), L-α-phosphatidylethanolamine (brain, sodium salt), azelaic acid (soybean phosphatide extract, granulated, 45% phosphocholine content), and 1,2-dimyristoyl-sn-glycerol-3-phosphatic acid were purchased form Avanti Polar Lipids, Inc., Birmingham, AL. Tolbutamide and chloropropamide were from Sigma, and glycine cyclic phosphate was from Aldrich. Lyophilized amylin was dissolved in deionized water at a concentration of 2 mg/ml, distributed into 25-μl aliquots, and stored at −20 °C. The peptide was thawed before addition to the lipid bilayer membranes and never frozen again.

**Planar Lipid Membrane Experiments—**Planar lipid bilayer membranes were formed as described previously (18) from 15 mg/ml solution of lipids in n-heptane at the end of Teflon tubing 250 or 500 μm in diameter. The construction of the chamber allowed substitution of the solution in one compartment (cis, to which peptides were added) within several seconds. After the membrane had turned black, the solution in the cis-side was replaced with amylin-containing solution. Usually, after the initial incorporation of amylin, free peptide in the aqueous solution was washed out. Membranes used in experiments were stable and had conductances of less than 10 picoeimhans up to voltages of ±100 mV for a period of at least 10 min prior to amylin addition.

**Recording Equipment—**Voltage clamp conditions were employed, and contact with the aqueous phases was made using Ag/AgCl electrodes with agar salt bridges. Electrode asymmetry was always less than 1 mV. Membrane formation was verified by monitoring membrane capacitance and resistance. Data were digitized and stored on VHS tape and played back for later analysis. An Axopatch 1C amplifier with headstage CV-3B was used for measuring membrane current. For data acquisition, a digital tape recorder and video cassette recorder allowed recording of large amounts of data. A storage oscilloscope was used for monitoring membrane capacitance and single-channel recordings. The cis-solution was taken as the virtual ground and the sign of the membrane voltage corresponded to the trans side of the membrane.

**RESULTS**

Human amylin peptide at concentrations ranging from 1 to 10 μM dramatically increased the conductance of pure planar lipid bilayer membranes (Fig. 1). At identical concentrations, nonamyloidogenic and noncytotoxic rat amylin produced no change in membrane conductance.

The amylin-induced conductance showed steady-state voltage-dependent behavior (Fig. 2). At negative voltages, the conductance remained stable. At positive voltages, it turned off during the first 3–5 min after a stepwise increase in voltage from 0 to values greater than +10–20 mV. The percentage of decrease in conductance was dependent on the amplitude of...
Membrane potential was initiated at soybean phospholipids (azolectin). Aqueous salt solutions contained 100 mM KCl, 5 mM Tris-HCl, pH 7.5. Membrane potential was switched to zero and then to −50 mV. This reversal of the sign of the membrane potential resulted in a fast increase in membrane current (channel “opening”). Returning to the original membrane holding potential (+50 mV) turned off the current to approximately its original level. A membrane with a diameter of 500 μm was made of a 15 mg/ml solution of phosphatidylcholine:phosphatidylserine:phosphatidic acid, 3:1:1 in heptane. Salt solutions contained 10 mM KCl, 5 mM Tris-HCl, pH 7.5.

Addition of 6 μM rat amylin (first arrow) to the aqueous phase dramatically increased the membrane current. The black lipid membrane was composed of soybean phospholipids (azolectin). Aqueous salt solutions contained 100 mM KCl, 5 mM Tris-HCl, pH 7.5. Membrane potential was initiated at +50 mV, to test stability, and then switched to −50 mV for the rest of the experiment.

The conductance induced by human amylin is due to the formation of ion-permeable channels (Fig. 4A). These channels exhibited a single channel conductance of approximately 7–8 picosiemens in 10 mM KCl (Fig. 4B). The single channel current jumps were quite uniform in size. At least four distinct levels can be discerned in this tracing. The conductance of the open state channel was ohmic (Fig. 4C). This suggests that the voltage dependence observed in macroscopic currents reflects a voltage-dependent probability of channel inactivation rather than a change in single channel conductance as a function of voltage. Although amylin clearly induces voltage-dependent conductance in 100 mM KCl, clean single channel recordings were difficult to obtain in this solution, possibly due to the very rapid rate of channel opening and closing in 100 mM KCl, or to the tendency of amylin to aggregate rapidly in higher salt.

The ionic selectivity of amylin channels was relatively poor. The channels exhibited a reversal potential of 14 mV (cation selective) in an 8-fold gradient of NaCl. Other selectivity experiments indicated that the channel is permeable to Na⁺, K⁺, Ca²⁺, and Cl⁻.

The dependence of membrane conductance on the concentration of amylin in the aqueous solution was linear (Fig. 5). This dependence suggests that a monomer or an amylin polymer, preexisting in the aqueous phase, interacts with the membrane and forms the channel. Amylin increased the lipid bilayer conductance in potassium chloride or sodium chloride at salt concentrations ranging from 10 mM to 1 M and at pH values ranging from 4.0 to 9.0. Whereas amylin channel-forming activity (number of channels incorporated into the membrane at a given peptide concentration and
time) was not dependent on pH, it was dependent on salt concentrations. Higher salt concentrations caused a decrease in channel-forming activity. As shown in Fig. 6A, the channel-forming activity of amylin in salt solutions composed of 10 mM KCl was more than 100 times higher than that in 1 M KCl.

Lipid composition of bilayer membrane was also found to play a role in the channel-forming activity of the peptide (Fig. 6B). Amylin exhibited the highest activity in membranes composed of a mixture of phosphatidylcholine/phosphatidylserine/phosphatidic acid, 3:1:1 (w/w/w). These membranes contained approximately 40% negatively charged lipids and therefore carried a high net negative surface charge. Thus, these mem-

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**Fig. 5.** Membrane conductance as a function of amylin concentration. Data are plotted from numerous experiments, indicating that the amylin-induced conductance is directly proportional to the concentration of human amylin peptide in the aqueous phase surrounding the membrane. Each point on the graph corresponds to the average measurements from three experiments. Membranes with a diameter of 500 µm were made from azolectin. Aqueous solutions contained 100 mM KCl, 10 mM Tris-HCl, pH 7.5.

**Fig. 4.** Single channel currents induced by human amylin. A, current trace is shown as membrane was held at −70 mV, to which human amylin had been added to a final concentration of 3 µM. Note the uniformity of single channel current size. The solvent-containing membrane was composed of soybean phospholipids (azolectin). Aqueous salt solutions contained 10 mM KCl, 3 mM Tris-HCl, pH 7.4. B, a histogram of single channel conductance sizes. Data are taken from the membrane depicted in A. “Events” were counted by observing the initial insertion into the membrane of channels, and thus each of the observations represents an independent channel event and not merely opening and closing of the same channel. C, the current-voltage relationship for the open state of the channel is linear.

**Fig. 6.** Dependence of amylin channel forming activity on ionic strength and lipid composition. A, membranes were composed of azolectin, and salt concentration was varied. B, measurements were carried out in constant salt solutions containing 10 mM KCl, 3 mM Tris-HCl, pH 7.5; lipid composition of membranes were varied as shown. The solid bars correspond to the mean of three to five measurements. Error bars show the standard deviation. Painted membranes with a diameter of 500 µm were used in all experiments. Channel forming activity of amylin was calculated as the number of channels incorporated into the membrane during 10 min following peptide addition to a final concentration of 5 µM.
branes were approximately 6 times more sensitive to amylin than membranes of pure soybean phospholipids (azolectin), which are composed of approximately 20% negatively charged lipids. Membranes composed of azolectin with cholesterol (cholesterol makes membranes more rigid) as well as those composed of diphytanoylphosphocholine (lipid head group net charge is zero) were only slightly sensitive to amylin.

In experiments directed toward possible modulators of amylin membrane activity, compounds related to Type II diabetes such as glucose (30 mM), tolbutamide, chlorpropamide, and glybenzycamide (2 mM) were studied. No modulation of amylin channel-forming activity nor change in amylin-induced ion currents was found after exposure to these compounds.

**DISCUSSION**

Our results demonstrate that human amylin interacts with membranes and is capable of ionic channel formation in lipid bilayers. The highly homologous rat amylin, which differs from human amylin at only 6 amino acid residues, did not form channels in planar lipid bilayers at comparable concentrations, indicating that it is unlikely that proteins of this general length and sequence form channels. The concentrations of human amylin used to obtain channel activity are quite comparable with the concentrations of other cytotoxic peptides, such as defensins (19), Aβ (20), Aβ 25–35 (21), magainins (22), cecropins (23), and sarcotoxins (24), needed to form channels in bilayer membranes. The facts that amylin channels can form in different lipid mixtures, are quite stable, are irreversibly associated with the membrane, and are all uniform in size argue strongly that they are not the result of nonspecific membrane disruption or peptide-induced defects in bilayer structure.

The total net charge of human amylin peptide is +5. Therefore it is not surprising that amylin had a higher channel forming activity on bilayers composed of negatively charged phospholipids and that increasing net lipid surface charge from 20 to 40% caused a significant increase in activity. Decreased channel formation at high salt concentrations can be explained by the screening of the membrane surface negative charge at high ionic strength solutions.

The voltage dependence of amylin induced ion currents is consistent with the fact that amylin has a net positive charge. In this case, the opposite negative voltage can help "drive" amylin across the membrane, transforming amylin into the ion conducting transmembrane conformation. However, since channels can insert into the membrane in a "closed" (low conductance) state at "closed" voltages (Fig. 3), the insertion process must be driven by nonelectrostatic forces.

Several lines of evidence suggest that channel formation may explain the cytotoxicity of amylin. 1) Channel formation occurs at concentrations comparable with those required for cytotoxicity (9). 2) The relatively poor selectivity of amylin channels would tend to lead to disruptions of ionic homeostasis, including influxes of Ca\(^{2+}\) and Na\(^+\) and effluxes of K\(^+\) and other vital cellular constituents. Prolonged elevations of intracellular Ca\(^{2+}\) levels, for example, may lead to cellular damage and even death (apoptosis) (25, 26). In addition to the potentially serious effects of these ionic changes, the cell would face increased energy demands as various pumps and exchangers attempted to compensate for these ionic disturbances. 3) Amylin channels exhibit voltage dependence, which would tend to keep channels in the open state at typical cellular transmembrane voltages. 4) The fact that human amylin forms channels and rat amylin does not is highly suggestive, since human amylin is toxic and amyloidogenic and rat amylin possesses neither property (9). 5) Amylin not only kills β cells but is able to kill other cell types. This relative nonspecificity is reflected in the fact that amylin can form channels in a planar lipid bilayer lacking any proteinaceous receptor. 6) It has been shown that human amylin interacts with phosphatidylycholine membranes and adopts β-structure (17). In contrast with human amylin, rat amylin did not change conformation upon addition to phosphatidylycholine membranes. The β-pleated sheet structure adopted by human amylin upon interaction with membranes is consistent with the β-structure found in the neurotoxic and channel-forming peptide Aβ25–35 (27) and is consistent with the β-structure seen in the bacterial porin (28) and mitochondrial outer membrane channel VDAC (29). 7) The amount of amyloid found in pancreatic islets is proportional to the amount of β cell destruction and to the insulin requirements of the patient (6, 30). Thus increased amylin deposition may lead to increased channel formation and β cell destruction, thereby increasing the insulin requirement of the patient.

We did not observe the requirement of actual contact between the membrane and amylin fibrils that was reported by Lorenzo et al. (9). This may reflect a requirement in the cellular system for a very large membrane leak in order to obtain cytotoxicity, due to the fact that β cells do not have a very tight membrane (membrane resistance, 10\(^8\) ohms (31)). Since the channels we have observed have a conductance of 7.5 picoSiemens in 10 mM KCl, it would take approximately 100 channels to make a significant (doubling the conductance) leak in these membranes (assuming a single channel conductance of approximately 100 picoSiemens in physiologic solution). Thus, it may be that islet cell dysfunction and destruction in vivo is a very slow, gradual process that builds up over considerable lengths of time. Blockers and inhibitors of channel activity may have potential therapeutic value in Type II diabetes.

Whole cell patch clamp recording from β cells has demonstrated that the addition of amylin induces hyperpolarization of the cell membrane and increases membrane current (32). Our present results predict a depolarizing effect on β cells. Since the patch clamp results were obtained at lower amylin concentrations (1–500 nM), they may represent a specific amylin interaction with an ion channel already present in the β cell membrane.

Several cytotoxic, amyloid- forming peptides have now been demonstrated to form ion-permeable channels (20, 21, 33, 34). While this may be coincidental, a more interesting view is that the structural properties of peptides that form β-sheet and aggregate into fibrils suit these peptides for membrane insertion and channel formation.

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