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EFFECT OF ANGIOTENSIN II ON ARTIFICIAL LIPID MEMBRANES

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Summary

(5-Isoleucine)-angiotensin II applied to black lipid membranes produced current fluctuations varying between $\Delta G = 5 \cdot 10^{-11}\ \Omega^{-1}$ and $3.5 \cdot 10^{-10}\ \Omega^{-1}$. These fluctuations depend on the voltage and the hydrostatic pressure. The membrane resistance is lowered by $\Delta R = 6.1 \cdot 10^{7}\ \Omega \cdot cm^2$. With (5-isoleucine, 8-leucine)-angiotensin II the jumps are of a single amplitude ($\Delta G = 2 \cdot 10^{-10}\ \Omega^{-1}$). In both cases water and ions are transported across the membrane.

The so-called ionophoric antibiotics are characterized by the property of transporting ions, water and neutral molecules across membranes; among them, the channel or pore-forming ones are thought to produce stable transmembrane structures that could mediate ion conductance in "liquid" as well as "solid" bilayers [1]. One of the most conspicuous manifestations of pore formation is the appearance of discrete conductance steps in an artificial black lipid membrane, which suggests the opening of individual channels. Several of the pore-forming antibiotics are short cyclic polypeptides, as in the classical example of alamethicin, or linear polypeptides, as in the case of the gramicidins (for reviews, see refs. 2, 3). For example, in the presence of very small amounts of gramicidin A, the current across a voltage-clamped black lipid membrane fluctuates between discrete and constant values with conductance jumps of about $4 \cdot 10^{-11}\ \Omega^{-1}$ [4]. These findings have led to the study of the effect of natural polypeptides on artificial membranes. Fettiplace et al. [5] observed that lysine-vasopressin increased the conductance of artificial bilayers, but they did not describe the formation of typical pores.

In the present work we have studied the interaction of the octapeptide hormone angiotensin II on black lipid membranes and have observed the

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production of typical conductance fluctuations, characteristic of a pore-forming ionophore. These changes are accompanied by the translocation of ions and water across the membrane.

Black lipid membranes were made according to Mueller et al. [6] on a 0.9 mm hole traversing a Teflon septum that separates two aquous phases. Ag-AgCl electrodes were used to apply a definite voltage across the membrane. Measurements of conductance were done by means of a Keithley 610C microammeter. A storage oscilloscope (Tektronix 5103 N), connected to a preamplifier (Philbrick 1029) and a Keithley inscriptor device were used for recording the voltage and current changes.

The membranes were made in an horizontal position, using a 2% solution of egg lecithin (Sigma) in decane. The bathing solution on both sides of the membrane contained 100 mM NaCl or KCl with 1 mM Tris/PO_4 buffer, pH 7.3. The drugs in 10—25-μl aliquots were applied on one side near the membrane.

(5-Isoleucine)-angiotensin II and the homologue (5-isoleucine, 8-leucine)-angiotensin II, kindly provided by Prof. A.C.M. Paiva (Escola Paulista de Medicina, 04023 Sao Paulo, Brazil) were synthesized by the solid phase method. The amino acid sequence of (5-isoleucine)-angiotensin II is: Asp-Arg-Val-Tyr-Ile-His-Pro-Phe; in (5-isoleucine, 8-leucine)-angiotensin II, the phenylalanine in the 8th position is replaced by leucine.

The addition of (5-isoleucine)-angiotensin II produced discrete changes in membrane conductance, while keeping the membrane clamped at a constant potential. In Fig. 1, a short time after the addition of 25 μl of a 0.1% solution of the polypeptide, typical current jumps appear with several levels of conductance Most of these have a value of $\Delta G = 5 \cdot 10^{-11}\,\Omega^{-1}$, but there are others of higher amplitude, which may reach $3.5 \cdot 10^{-10}\,\Omega^{-1}$. In this particular case there were

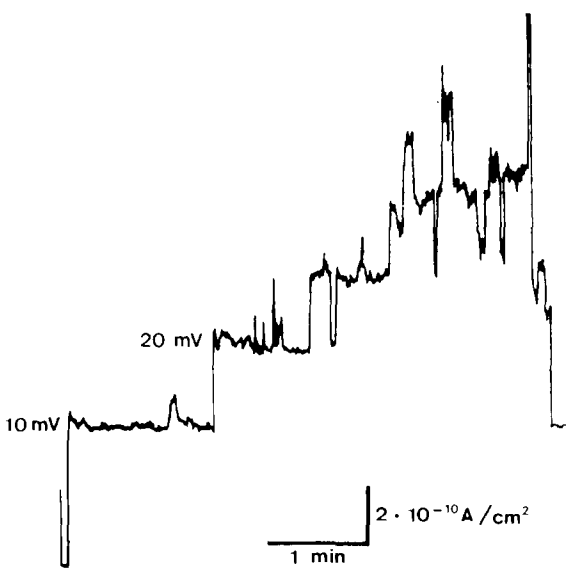

Fig. 1. Trace of current versus time. Voltages of 10 and 20 mV are applied across a membrane formed with a 2% lecithin-decane solution. Angiotensin II (5-isoleucine) was added near the membrane. The smallest conductance changes correspond to $\Delta G = 5 \cdot 10^{-11}\,\Omega^{-1}$.

no fluctuations at 10 mV, but they appeared at 20 mV. Such current fluctuations appear also to be related to the hydrostatic pressure applied to the membrane. If 0.5 ml excess solution (which corresponds to $2.6 \cdot 10^{-5}$ atm) is applied on one side, then the first pores appear 2 min after the addition of angiotension II. When there is no pressure applied, it may take 20 min for the pores to show up and only when higher potentials (80—100 mV) are applied. Membranes, undergoing interaction with angiotensin II-(5-isoleucine) for 30 min, lowered their resistance from $R_1 = 6.7 \cdot 10^7\,\Omega \cdot \mathrm{cm}^2$ to $R_2 = 5.6 \cdot 10^6\,\Omega \cdot \mathrm{cm}^2$ ($\Delta R = 6.1 \cdot 10^7\,\Omega \cdot \mathrm{cm}^2$) Simultaneously there was the building up of a hydrostatic pressure in the upper chamber. In this case the membrane showed a concavity looking upwards. If the pressure is equilibrated by removal of liquid from the upper compartment, the membrane flattens, the pores disappear and the initial membrane resistance is restored. This phenomenon is reversible and pores can be seen again after application of a hydrostatic pressure. With time, no membrane potential could be detected, suggesting that the pores are not ion selective and that they probably allow the passage of Na^+ and Cl^- as well as water. With KCl in the bathing solution similar current jumps could be observed; however their frequency was lower and also the decrease in resistance of the membrane was smaller than with NaCl ($\Delta R = 1.1 \cdot 10^7\,\Omega \cdot \mathrm{cm}^2$).

The effect of (5-isoleucine, 8-leucine)-angiotensin II on the artificial lipid membrane was slightly different. Immediately after the application of the polypeptide, pores having a $\Delta G = 2 \cdot 10^{-10}\,\Omega^{-1}$ appeared. As shown in Fig. 2 these pores in general are of a single amplitude and very seldom different levels of conductances were observed; also here the hydrostatic pressure and the resistance of the membrane changes with time.

In the case of the pore-forming antibiotics it has been postulated that the channels result from the special organization of the molecules within the bilayer. Transmembrane structures are thought to be formed of repeating

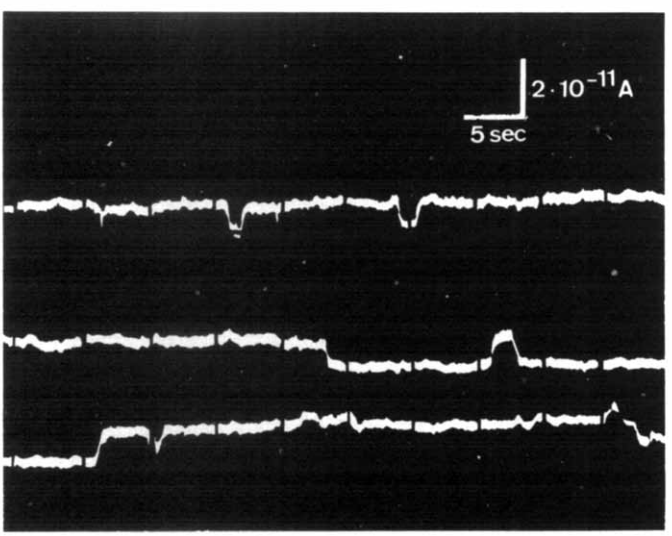

Fig. 2. Trace of current versus time photographed from the oscilloscope. 40 mV were applied across the membrane made with a 2% lecithin-decane solution. Angiotensin II (5-isoleucine, 8-leucine) was added upon one side of the membrane. Conductance changes correspond to $\Delta G = 2 \cdot 10^{-10}\,\Omega^{-1}$.

units which may arrange in series or in parallel (i.e. as the staves of a barrel) and which may exhibit a dependence on the electrical field across the lipid bilayers. Bauman and Mueller [7] have proposed that the assembly of a channel is made by the voltage-dependent aggregation of monomeric precursors. According to this hypothesis the gating mechanism for the channel involves the voltage-induced insertion of the precursor from the membrane surface into the hydrocarbon region of the bilayer [8].

Juliano and Paiva [9] and Deslauriers et al. [10] have discussed the several molecular models that have been proposed for angiotensin II. From a carbon-13 spin-lattice relaxation study Paiva and co-workers conclude that (5-isoleucine)-angiotensin II is probably folded so that the N- and C-terminals are held in closer proximity than expected for a random conformation. The molecular model that emerges is that of a molecule folded on itself and having a radius of about 8 Å. The (5-isoleucine, 8-leucine)-angiotensin II which has only 1% of the physiological activity of the other, showed a 10% increase in spin relaxation time, indicating a loosening of the conformation in the backbone of the molecule.

The present study shows that both polypeptides of angiotensin II are able to interact with lipid bilayers made of lecithin to create pores. We may speculate that the interaction of these polypeptides with the lipid bilayer leads to a rearrangement of several of these folded molecules, so that the more hydrophobic parts of the chains are held toward the lipidic milieu, while the hydrophilic groups face towards the center of the channel. The relative wide diameter of the pore formed may explain the flux of water and the non-ionic selectivity. As in the case of other pore-forming polypeptides the gating mechanism for angiotensin II would be electrically driven. As shown in Fig. 1 the pores are mainly opened under an applied positive potential. Another factor that appears to be involved in the gating mechanism is the hydrostatic pressure that produces a stretching of the membrane; such a pressure may be externally imposed upon the membrane or it may be built by the preferential flux of water and ions toward the compartment containing the polypeptide.

While the two homologues of angiotensin II used in the present study are able to produce channels, the (5-isoleucine, 8-leucine)-angiotensin II seems to be more active and this could be related to the more relaxed conformation of this molecule [10].

The observations reported here suggest that the octapeptide angiotensin II may have a direct interaction with the lipid bilayers of the membrane. However, the relevance of this interaction in the mode of action of the hormone in the biological system, in which a specific receptor is involved, remains open to further investigations.

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