CONFORMATIONAL CHANGES AT MEMBRANES OF TARGET CELLS INDUCED BY THE PEPTIDE HORMONE ANGIOTENSIN. A SPIN LABEL STUDY*

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1. Introduction

The mechanism of action of many hormones is thought to be based on the interaction between the hormone and the membranes of target cells. We have examined the interaction of AII** with membranes from smooth muscle of guinea pig ileum [1] through binding studies with the radioactive hormone and monitoring of conformational changes at the membranes with the spin label technique [2,3].

2. Experimental

2.1. *Materials*

The peptides were prepared by the solid phase method [4]. [^{3}H]AII was a gift from Dr P. A. Khairallah from the Cleveland Clinic, Ohio, and had a specific activity of 40 Ci/mmole. Spin labels were from Syva, Palo Alto, Calif. USA. Phospholipids were from Lipid Products, South Nutfield, England.

2.2. *Binding experiments*

Aliquots of freshly prepared subcellular fractions [1] (1–2 mg protein) were incubated for 10 min at 37°C with [^{3}H]AII (final concentration 10^{-8} M) in Tyrode solution. GSWP Millipore filters were used to separate bound from free AII. Radioactive counting of the filters washed with buffer was done with a Beckman LS 100 scintillation counter. Correction for trapping or adsorption of angiotensin to the filters in the absence of cell fractions was effected. 'Specific' binding [5] was obtained subtracting the amount that was not displaced by native 10^{-5} M angiotensin II from the total radioactive uptake.

Protein was determined by the method of Lowry et al. [6]. Lipids were extracted according to Folch et al. [7].

2.3. *Spin label experiments*

2.3.1. Plasma membranes

Membranes (0.8–1.5 mg protein/ml) were suspended in Tyrode solution, pH 7.5. The incorporation of MSL-6 was performed at 4°C, for approx. 12 hr, with 0.010–0.013 mg label/mg membrane protein. 5-SASL, at ratios of 0.003–0.0125 mg/mg membrane protein, was intercalated by three different procedures (refs. [8–10]). The spectra, taken after 3–4 washings, did not depend on the method of probe intercalation. Some preparations with higher lipid probe concentration displayed some exchange broadened spectra. Samples were placed in Pasteur pipettes.

2.3.2. Model systems

Lipid dispersions and planar multibilayers were prepared as described in ref. [11]. Flat aqueous cells (James Scanlon, Costa Mesa, Calif. USA) were used. Spectra were taken with a Varian E-4 esr spectrometer at room temperature (22 ± 2°C).

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** Abbreviations: AII, angiotensin II; [^{3}H]AII, tritiated AII, MSL-6, 4-maleimido-2,2,6,6-tetramethyl-piperidinooxyl; 5-SASL, 2-(3-carboxypropyl)-4,4-dimethyl-2-tridecyl-3-oxazolidinyloxyl.

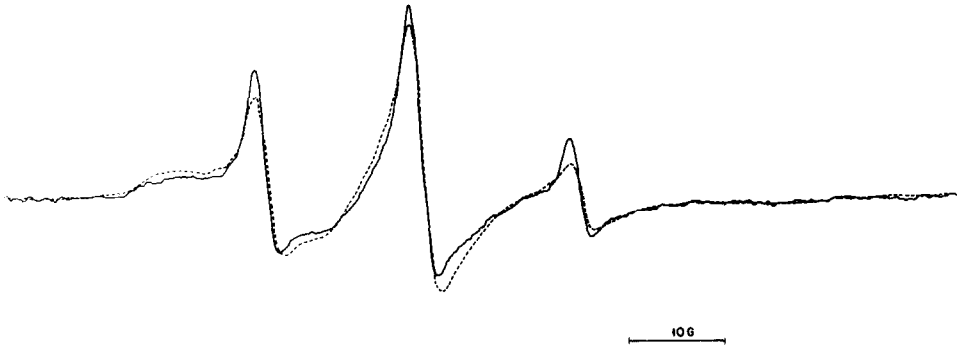

Fig. 1. Spectra of MSL-6 in plasma membrane from smooth muscle of guinea pig ileum suspended in Tyrode solution. Solid line – in the absence of AII; dotted line – in the presence of a 1 mM solution of AII. Experimental conditions are given in the text.

3. Results

3.1. *Binding experiments*

The specific binding of [^{3}H]AII to the plasma membrane fraction was 160 pmoles/g protein, while mitochondria bound only 65 pmoles/g protein. No binding to microsomes was detected. Therefore, only plasma membranes were used for the spin-labelling experiments.

3.2. *ESR studies with the protein-specific label*

Membranes containing MSL-6 displayed spectra with two components: one characteristic of label bound to sites of higher mobility, and another indicative of sites where the motion of the label was greatly restricted [2] (fig. 1). Addition of a 1 mM solution of AII produced an increase in the population of label in a more hindered environment (fig. 1).

3.3. *ESR studies with the lipid spin probe*

3.3.1. Intact membranes

Spectra of the stearic acid probe in aqueous suspensions of membranes also indicated that it was in two different states (fig. 2): one of a high degree of mobility, and another where motion was hindered (membrane-bound probes). It was difficult to measure accurately the separation between the outer extrema [12] since the spectrum presented a very broad high field line (fig. 2). Therefore we did not use changes in this parameter to monitor the effect of the hormone. The narrow lines have already been observed in other membrane preparations [8–10, 13–15]. They seem to be due to probes tumbling more freely in an aqueous

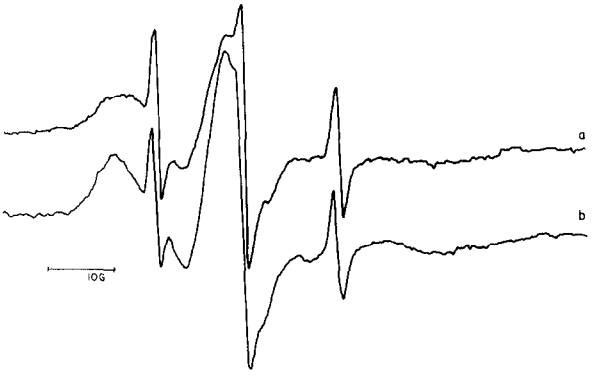

Fig. 2. Spectra of 5-SASL in plasma membranes from smooth muscle of guinea pig ileum suspended in Tyrode solution. a) in the absence of AII; b) in the presence of a 1 mM solution of AII. Experimental conditions are given in the text.

environment, as measured by the hyperfine splittings and linewidths (ref. [16] and our own observations). A decrease in their intensity occurred in presence of the hormone (fig. 2b). Addition of AII (final concentration 1 mM) to an aqueous solution of 5-SASL caused no spectral changes. The effect of angiotensin was concentration dependent (table 1). The effect was smaller for 1 mM AII when such concentration was added to preparations previously treated with 0.01 mM hormone, in a way that ressembled the phenomenon of tachyphyllaxis, characteristic of the action of AII on the guinea pig ileum [17].

The alterations promoted by two analogs, Leu^8-AII and Gly^1,Pro^8-AII, occurred to a significantly smaller extent (table 1).

Table 1
Effect of angiotensin II and analog peptides on the spectra of 5-SASL-labelled membranes

Exp.*	(h_m/h_f)** (no peptide added)	Peptide added	Conc. (mM)	h_m/h_f** (after addn. of peptide)	% change
1	0.420	AII	1.00	0.840	100
2	0.437	AII	1.00	0.840	92
3	0.228	AII	0.10	0.375	65
4	0.409	AII	0.10	0.623	52
5	0.256	AII	0.01	0.281	10
6	0.344	Leu^8-AII	1.00	0.448	30
7	0.465	Gly^1,Pro^8-AII	1.00	0.598	29

* The experiments described in this table are representative of a large number of observations.
** h_m/h_f is the ratio of the heights of the low field lines corresponding to membrane-bound (h_m) and freely tumbling (h_f) spin probe.

3.3.2. Delipidated membranes

Delipidated membranes retained some of the lipid probe, yielding spectra which also presented the narrow lines. The relative intensity of the latter also decreased upon addition of AII.

3.3.3. Lipid extracts and model systems

The spectra yielded by aqueous dispersion of membrane lipids underwent no changes when AII was added. The same was true for films and dispersions of known lipid composition.

4. Discussion

Our preliminary results with radioactive angiotensin show a higher affinity of the hormone for plasma membranes than for the other subcellular fractions of the intestinal smooth muscle. This binding, observed with the hormone concentration needed for half maximum contraction in the intact ileum [8], could be displaced by native angiotensin. These findings are in agreement with previous work with angiotensin receptors in rabbit aorta [19]. This, and results obtained with bovine and rat adrenal glands [20], corroborate the current view that certain hormones interact first with the plasma membranes of target cells.

Binding may be a first step, leading to conformational changes at the membrane as part of the mechanism of hormone action.

The changes in the spectra of spin labels covalently bound to plasma membrane proteins indicate that the hormone caused a decreased mobility of a number of sites. These results are in line with the finding that the hormone caused contraction of the isolated plasma membranes [1]. However, the diminished intensity of the narrow lines in the spectra of 5-SASL upon addition of the hormone suggests an enhancement of the fluidity of membrane lipid regions, and, as a consequence, an increased 'solubility' of spin probes in the membrane. We ascribe this event to the small lipid: protein ratio (1:3), of our membranes. It is conceivable that the membrane lipids would tend to expand in order to accomodate for protein contraction, but since there is a large proportion of protein, the overall result is the observed contraction [1]. The fact that delipidated membranes gave the same results as intact membranes might be explained by the presence of some lipid not removed during the delipidation procedure which would display the same behaviour as the total lipid. Nevertheless, it cannot be ruled out that the relative decrease in the intensity of the lines due to more freely tumbling 5-SASL might be caused by a tight binding of the probe to membrane proteins as a result of the interaction with angiotensin. That the site of action of the hormone must be protein in nature was shown by the absence of an effect in systems containing only lipids.

Effects that were qualitatively similar to those observed with AII were also seen both with Leu^8-AII (intrinsic activity 48% that of AII and pD_2* = 5.00, as compared with pD_2 = 8.80 for AII [17]) and with Gly^1–Pro^8-AII (intrinsic activity 96%, pD_2 = 4.00 [21]). However, the effect was consistently and significantly more pronounced with AII (table 1). This, and the tachyphyllactic behaviour, suggest that a significant part of the membrane conformational changes evidenced by the spin label method may be connected with the activation of the effector system that follows the binding of AII to its receptor site. Conformational alterations at membranes have been detected with lower concentrations of other hormones [22–24] than in our experiments. One possible explanation for the high AII concentrations needed to produce the changes reported here may be that a partial loss of the effector system occurred during the preparation of the membranes.

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* pD_2 is the negative logarithm of the drug-receptor dissociation constant, taken as the concentration needed to produce half maximum response.