Effect of insulin on lateral diffusion of pyrene in rat liver plasma membrane

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Summary. The yield of excimer formation by pyrene molecules inserted in rat liver plasma membranes is sensibly decreased in the presence of 1 nM insulin. This effect can be interpreted as indicating a decrease of the value of the translational diffusion coefficient of the dye within the membrane.

The interaction of insulin with appropriate receptors on the plasma membrane of target cells appears to be the first step in the action of the hormone[3-5]. More recently it has been suggested, on the basis of the behaviour of membrane-bound enzymes, that insulin may induce, as it binds to the plasma membrane, a decrease in membrane fluidity[6,7]. This hypothesis has received further support from studies[8] in which the lipid microviscosity of rat liver plasma membrane was estimated from the fluorescence polarization of the probe 1,6-diphenyl-1,3,5-hexatriene according to Shinitzky and Inbar[9]. Since however the validity of this assay has been questioned by a number of authors[10,11], mainly on the basis of the anisotropic nature of the membrane environment surrounding the probe, we have tried to verify, by an independent method, whether insulin specifically modified lateral diffusion phenomena in the plane of the hepatocyte plasma membrane.

Since it is well-known that photoexcited molecules of the fluorescent aromatic hydrocarbon pyrene can, in nonpolar media, form collisional complexes ('excimers') with other unexcited pyrene molecules[12], we made use of this property to investigate whether insulin modifies the frequency of excimer formation by pyrene molecules inserted in rat liver membranes.

Materials and methods. Liver plasma membranes were obtained from male Sprague-Dawley rats, as previously reported[13], and resuspended in Tris-buffered saline (50 mM Tris-HCl, pH 7.5, 100 mM NaCl), the final membrane protein concentration in the assay being about 60 µg/ml.

The membrane suspension was incubated at 25 °C for 1 h, in the presence of 1 nM bovine insulin (from B.D.H., Poole, England), or of 25 µg concanavalin A/ml (from Miles-Yeda, Rehovot, Israel), or without any addition. Incorporation of pyrene into the membranes was subsequently accomplished by adding, to the aqueous membrane suspension, a concentrated ethanolic solution of the dye (the final alcohol concentration being less than 0.4%, v/v). Distribution of pyrene into the membranes occurred within the mixing time.

Pyrene fluorescence spectra were recorded, using 342 nm as the excitation wavelength, on a FICA model 55 L spectrophotofluorimeter, the spectra being corrected in terms of incident energy. The emission spectra showed 'monomer' bands at 376 and 394 nm, and an excimer band centered at 480 nm; in the absence of membranes the emission at 480 nm was very low.

As indicated by Vanderkooi and Callis[14], the rate of excimer formation can be analyzed in terms of the theory of diffusion-controlled fluorescence quenching reactions, as developed by Yguerabide et al.[15], pyrene molecules acting both as fluorophores and as quenchers. Measurement errors were found to be minimized if we evaluated, as a function of total pyrene concentration, the increase of excimer concentration, [d], rather than, as suggested, respectively, by Yguerabide et al.[15] and by Vanderkooi and Callis[14], the decrease of the excited monomer or the excimer/monomer ratio. If R is the interaction radius between 2 pyrene molecules (~10 Å), D is twice the translational diffusion coefficient of pyrene in the plane of the membrane, k_F^* is the sum of the radiative decay rates of the excited pyrene monomer (7.4×10^6 sec^{-1}), N′ is the Avogadro's number in mmoles (6.02×10^{20}), M the theoretical pyrene concentration (i.e. the known amount of pyrene added to the membrane suspension) and β the partition coefficient of pyrene between the membrane and the water phases - the M.β-product being therefore the effective concentration of the probe in the membrane - the concentration [d] of the excimer will be given by:

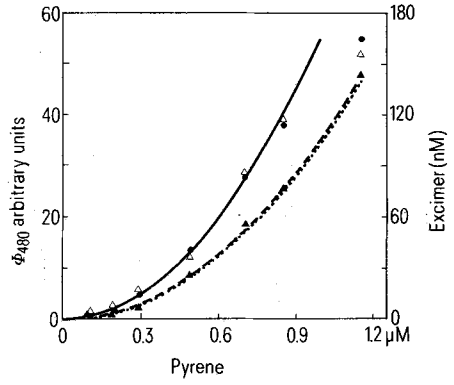
$$[d]=M.\beta \times\left\{1-k_F^*\times\frac{1-b\,(\pi/a)^{1/2}\cdot\exp\,(b^2/a)\,[1-\mathrm{erf}\,(b/a^{1/2})]}{a}\right\}, \quad (1)$$

where $a=k_F^*+4\pi\,RN'DM$ and $b=4\,R^2N'M\beta\,(\pi\,D)^{1/2}$.

The dependence of [d] from M, according to equation 1 and using suitable values for D and β, was estimated by using a Hewlett-Packard model 9830 table-top computer, equipped with an X-Y plotter H-P mod. 9830 A.

Results and discussion. In the figure are reported the intensities of fluorescence emission at 480 nm ('excimer peak') as a function of pyrene concentration in a rat liver plasma membranes suspension, in the presence (closed triangles) or absence (open symbols) of 1×10^{-9} M insulin. It can be seen that, up to approximately 1 µM pyrene, the excimer concentration is sensibly lower in the presence of the hormone than in the absence. Concanavalin A (closed circles) does not cause any modification of excimer formation.

A relatively good fit to the experimental data in the absence of insulin can be obtained, in this lower range of



Dependence of excimer yield upon pyrene concentration in rat liver plasma membranes. Open symbols represent the experimental results, evaluated as fluorescence intensity at 480 nm, obtained with the membranes as such; closed triangles those obtained in the presence of 1 nM insulin; closed circles those in the presence of 25 µg concanavalin A/ml. Each point is the mean of 3 separate experiments. The curves have been drawn on the basis of equation 1, using appropriate values of D and β: the solid line, with $D=2.8\times10^{-8}$ cm$^2\cdot$sec^{-1}, and $\beta=2\times10^4$; the dashed line, with $D=1.4\times10^{-8}$ cm$^2\cdot$sec^{-1}, and $\beta=2\times10^4$; the dotted line, with $D=2.8\times10^{-8}$ cm$^2\cdot$sec^{-1}, and $\beta=1.4\times10^4$. On the abscissae are reported the mean pyrene concentrations in the membrane suspension.

pyrene concentrations, by use of equation 1, assuming, for the various parameters, values similar to those found by Vanderkooi and Callis[14] (figure, solid line).

If the pyrene concentration is raised above 1 μM, the experimental points exhibit a negative deviation which cannot be accounted for by simple diffusion theory and has been attributed[14] to an alteration in membrane structure caused by insertion of several pyrene molecules.

In the presence of insulin, it can be easily seen that the experimental data can be simulated by decreasing the value of the translational coefficient D from 2.8×10^{-8} to 1.4×10^{-8} $cm^2 \cdot sec^{-1}$ (figure, dashed line) and/or by decreasing the value of the partition coefficient β (figure, dotted line). The latter of these 2 possibilities, i.e. that insulin decreases the solubility of the dye in the membranes, seems rather unlikely, since it would involve a gross and generalized effect of the hormone on the structure of the membrane lipid bilayer. The former possibility, instead, gives further support to the hypothesis[6-8] that insulin causes a decrease of membrane fluidity. The effect on lateral diffusion would even appear to be more marked than that on the overall lipid microviscosity observed by Luly and Shinitzky[8], possibly because the variations in the rate of pyrene excimer formation give specific information on the mobility in the plane of the membrane.

Due to the physiologically low hormone concentrations used, the effect observed upon addition of insulin appears not to be attributable to a nonspecific cross-linking of membrane receptors by insulin dimers; nor does concanavalin A duplicate the insulin effect.

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Endocrinological adaptations in insects

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Summary. Insect larvae respond differently to juvenoid treatment. Larvae occurring in relatively constant ecological conditions with abundant food, e.g. *Trogoderma granarium, Tribolium castaneum, Corcyra cephalonica* and *Ephestia cautella,* can undergo several extra larval moults, show growth with each moult, may moult 2–3 times with 1 treatment and do not suffer ecdysial failure. Larvae adapted to changing ecological conditions, e.g. *Spodoptera litura, Earias fabia, Papilio demoleus, Euxoa spinifera* and *Attacus ricini,* rarely undergo supernumerary moults, and the supernumerary instars suffer from ecdysial failure. It is suggested that the former have evolved an endocrinological adaptation.

We have studied the effect of feeding and topical application of some juvenoids on the larvae of two Coleopteran species viz. *Trogoderma granarium* and *Tribolium castaneum* and 7 Lepidopteran species viz. *Corcyra cephalonica, Ephestia cautella, Spodoptera* (Prodenia) *litura, Earias fabia, Papilio demoleus, Euxoa spinifera* and *Attacus ricini,* and we find that they can be divided into 2 categories on the basis of their response to the juvenoid treatment.

Larvae of 1 category, including *T. granarium, T. castaneum, C. cephalonica* and *E. cautella,* easily undergo repeated supernumerary larval moults when the last normal larval instar and successive supernumerary instars are topically treated with adequate quantities of the juvenoids, or when they are reared in food media containing adequate quantities of the juvenoids in the food throughout. Treated either way with appropriate quantities of the juvenoids, 100% larvae undergo supernumerary moults. *T. granarium* may thus undergo upto about 23 (in female) and 19 (in male) supernumerary larval moults, *T. castaneum* 13 supernumerary moults and *C. cephalonica* and *E. cautella* 22 supernumerary moults. Further, upto a certain number of supernumerary moults (about 15 in female and 12 in male in *T. granarium,* 8 in *T. castaneum,* 16 in *C. cephalonica* and in *E. cautella*), the larvae continue to grow in size, and, when removed from the hormone-mixed media or when topical treatment with the juvenoid is not given any more, they undergo a few supernumerary larval moults and then pupate and complete the metamorphosis to produce 'giant' pupae and adults. If treated longer with the hormone, though the super larvae may still continue to moult, they tend to become reduced in size and are unable to pupate and finally die. Moreover, in these forms, it is interesting to note that a single treatment with a juvenoid given to the normal last larval instar or a supernumerary larval instar may cause 2 or 3 extra moults to take place. Supernumerary larval instars in all these cases seldom show signs of ecdysial failure.

Larvae of the other category, including those of *S. litura, E. fabia, P. demoleus, E. spinifera* and *A. ricini,* undergo supernumerary larval moults with difficulty. With heavy and properly timed doses of the hormone given topically or via the gut, a small percentage of the larvae only may produce 1 *(S. litura, E. fabia, E. spinifera, A. ricini)* or rarely 2 *(P. demoleus)* supernumerary larval instars. The supernumerary larval instars are, as a rule, non-viable, do not pupate and usually suffer from ecdysial failure. If, rarely, a supernumerary larval instar does metamorphose, an abnormal adultoid is produced.

Supernumerary larval instars have been reported to be produced by juvenoid treatment in several other insects,