# THE BINDING OF POLYSOMES TO SMOOTH MEMBRANES OF RAT LIVER PROMOTED BY STEROID HORMONES AND EXTRACTS FROM EITHER ROUGH ENDOPLASMIC RETICULUM OR FROM POLYSOMES OF THE OPPOSITE SEX

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## 1. Introduction

The attached polysomes can be removed from the membranes of rough endoplasmic reticulum of rat liver by treatment with EDTA and the resultant 'degranulated' membranes will rebind polysomes in the presence of magnesium ions at physiological pH values [1, 2]. In sharp contrast, smooth membranes will not interact with polysomes under these conditions. It has recently been demonstrated [1, 3, 4] that the attachment of polysomes to smooth membranes can be achieved by adding a steroid hormone. The hormone requirements are totally sex specific, with oestradiol and testosterone as the most effective substances yet discovered for promoting polysome attachment to smooth membranes from male and female rat liver respectively.

We report in this paper the steroid hormone requirements for the interaction of membranes with polysomes from the opposite sex. We show that 'activating substances' can be obtained from polysomes by extraction with ethyl acetate which have remarkable sex specific effects. In the membrane–polysome binding systems the extract from female polysomes will replace oestradiol but not testosterone, and the extract from male polysomes, testosterones but not oestradiol. An extract from male rough endoplasmic reticulum will replace both hormones. The implications of these findings are discussed.

## 2. Experimental

### 2.1. *Membrane–polysome association*

This was determined by measuring the activity of a membrane-bound disulphide rearranging enzyme, the activity of which is masked when ribosomes are bound to the membranes [4, 5]. Some of the results were confirmed by measuring RNA attached to the membranes [5].

### 2.2. *Microsomal subfractions*

These were prepared from rat liver by the methods previously described [5] with the modification that the discontinuous sucrose gradient consisted of 10 ml of 1.2 M sucrose and 4 ml 1.7 M sucrose in TKM buffer (tris/HCl pH 7.5 (50 mM) containing KCl (25 mM) and $MgCl_2$ (5 mM).) Centrifugation was for 3½ hr at 78,000 *g*. The free polysomes, rough and smooth membranes were aspirated from the gradient, diluted with 0.25 M sucrose/TKM and harvested by centrifugation (78,000 *g* for 1¼ hr). If stored for less than three days the preparations were resuspended in 0.25 M sucrose/TKM and kept in an ice bath. For storage of up to two weeks the preparations were resuspended in 1 M sucrose/TKM and frozen.

### 2.3. *Degranulated rough membranes*

These were prepared by dialysis of rough membrane preparations at 2–4° overnight, against 200 volumes of tris buffer (tris/HCl pH 7.5, 50 mM) containing 25 mM KCl and 5 mM EDTA) followed by

separation either on a layered sucrose gradient (1.2 M sucrose TKM over 1.7 M sucrose TKM) or over 2 M sucrose TKM. The membrane-containing band was aspirated, diluted with 2–3 volumes of 0.25 M sucrose/TKM and harvested by centrifugation of 150,000 *g* for ¾ hr.

2.4. *Ethyl acetate extracts of subfractions*

The free polysomes or rough membranes from 2–4 rats were suspended in water (3ml) and shaken with ice cold ethyl acetate (6 ml) for 5 min. The mixture was centrifuged, the ethyl acetate layer removed and the extraction repeated twice. The ethyl acetate extracts were bulked and evaporated to dryness in vacuo. The residue was dissolved in dimethyl formamide (50 μl), diluted with 0.25 M sucrose/TKM (150 μl) and stored at 4°.

2.5. *Incubations of membranes with polysomes*

These were carried out at 32° at pH 7.5 (TKM buffer). The membranes were used at a concentration of 5–7 mg protein per ml and the polysomes in the range 2–5 mg RNA per ml.

The following were also added where stated: post-microsomal supernatant, to 20% final volume; ethyl acetate extracts, to 5% final volume; steroids, 5μg/ml (from a stock solution in dimethyl formamide of 1 mg per ml).

Aliquots of 100 μl were withdrawn at convenient time intervals and the activity of the disulphide rearranging enzyme determined. (Experimental details are in [6]).

## 3. Results

The steroid hormone requirements for the interaction of various combinations of male or female smooth membranes with male or female polysomes are shown in fig. 1. The data confirm the previous observations [3, 4] that male smooth membranes combine with male polysomes in the presence of oestradiol whereas female membranes combine with female polysomes in the presence of testosterone. It has already been demonstrated [4, 6] that testosterone is inactive in the male and oestradiol inactive in the female in this in vitro system. It can be clearly seen that the interaction of male membranes with female polysomes occurs in the presence of testosterone and not oestradiol; the interaction of female membranes with male polysomes requires oestradiol and not testosterone. These results suggest but do not prove that the pair of sex hor-

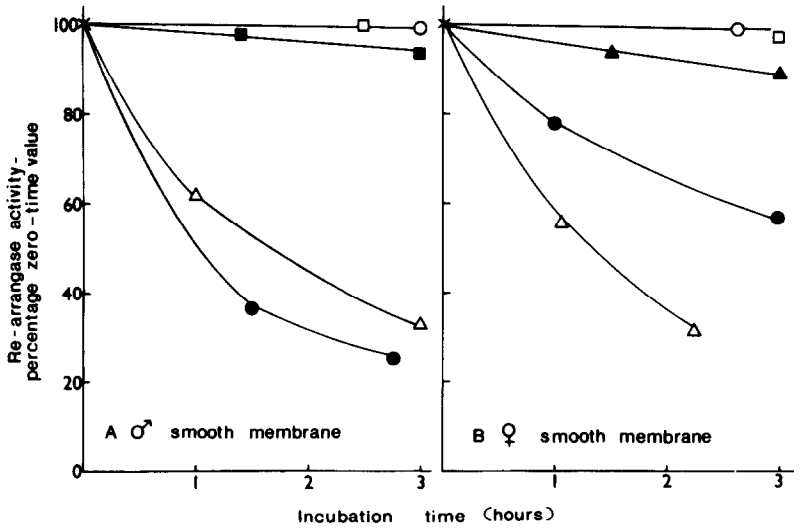


Fig. 1. Intercation of smooth membranes from male (A) and female (B) rat liver with polysomes. Membranes were incubated with the following components as described in the text. ○ male polysomes, no steroids; □ female polysomes, no steroid; ● male polysomes plus oestradiol; ▲ male polysomes plus testosterone; ■ female polysomes + oestradiol; △ female polysomes + testosterone. X zero time point for all experimental series. Female post-microsomal supernatant was added with the female polysomes, except □ in A. Male post-microsomal supernatant was added with male polysomes, except ○ in B.

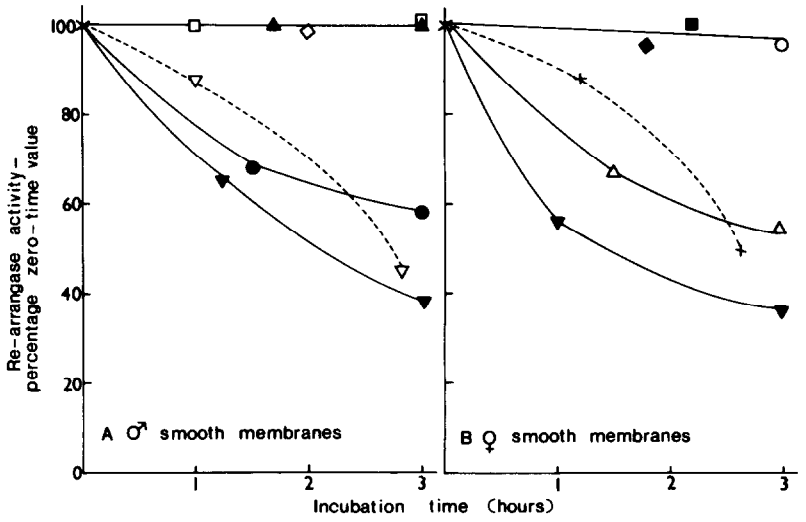


Fig. 2. Interaction of smooth membranes from male (A) and female (B) rat liver with polysomes. ○ male polysomes, no extract; □ female polysomes, no extract; male polysomes plus ethyl acetate extract of: ● female polysomes, ▲ male polysomes, ▽ male rough membranes; female polysomes plus ethyl acetate extract of: ■ female polysomes, △ male polysomes; ▼ male and female polysomes, no extract; no polysomes, ethyl acetate extract of: ◇ female polysomes, ◆ male polysomes; + female polysomes plus ethyl acetate extract of male rough membranes. X zero time point for all experimental series.

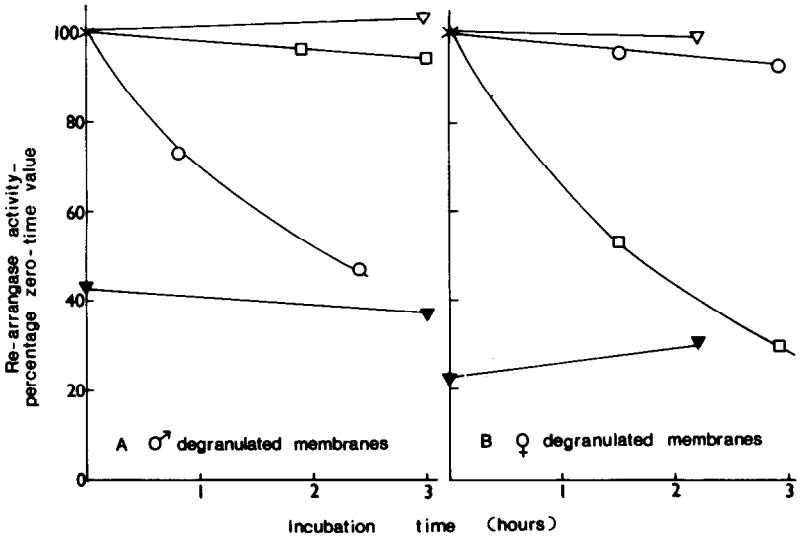


Fig. 3. Interaction of degranulated rough membranes from male (A) and female (B) rat liver with polysomes. Except where stated, degranulated membranes were incubated with the following components in the presence of post-microsomal supernatant from the same sex as the membranes. ○ male polysomes; □ female polysomes; ▽ no polysomes (control); ▼ rough membranes from which the degranulated membranes were prepared + polysomes of the same sex (control). X zero-time point for all experimental series.

Table 1
Requirements for the interaction of membranes with polysomes.

| Membrane Type | Sex | Polysomes (Sex) | Post-microsomal supernatant (sex) | Added material | Formation membrane–polysome complex |
|---|---|---|---|---|---|
| S | M | M | M | oestradiol | + |
| S | M | F | F | oestradiol | – |
| S | M | M | M | testosterone | – |
| S | M | F | F | testosterone | + |
| S | M | M | M (or none) | none | – |
| S | M | F | F (or none) | none | – |
| S | M | M+F | M+F | none | + |
| S | M | M | none | EP, M | – |
| S | M | M | none | EP, F | + |
| S | M | M | none | ER, M | + |
| S | F | F | F | testosterone | + |
| S | F | M | M | testosterone | – |
| S | F | F | F | oestradiol | – |
| S | F | M | M | oestradiol | + |
| S | F | F | F (or none) | none | – |
| S | F | M | M (or none) | none | – |
| S | F | M+F | M+F | none | + |
| S | F | F | none | EP, F | – |
| S | F | F | none | EP, M | + |
| S | F | F | none | ER, M | + |
| D | M | M | M | none | + |
| D | M | F | M | none | – |
| D | M | F | F | testosterone | + |
| D | F | F | F | none | + |
| D | F | M | F | none | – |
| D | F | M | M | none | – |
| D | F | M | F (or none) | oestradiol | +* |

(M, male; F, female; S, smooth membranes; D, degranulated rough membranes; EP, ethyl acetate extract of polysomes; ER, ethyl acetate extract of rough reticulum).
* by measurement of bound RNA only, one of a series of unpublished experiments by Mr. M.B. Cooper.

mones are involved in the membrane–polysome interaction. This would require that oestradiol and testosterone or equivalent substances are already present in the female and male polysomes since the steroid requirements for interaction of membranes with polysomes of the opposite sex preclude the presence of the hormone in the membranes.

The results shown in fig. 2 conclusively demonstrate that a material can be extracted from female polysomes which replaces the requirements for oestradiol. A similar extract from male polysomes will replace testosterone . The extract from rough endoplasmic reticulum will replace both oestradiol and testosterone. The extract from rough reticulum gives experimental curves which show an apparent induction phenomenon and appear to differ significantly in shape from the other curves. It is of interest that corticosterone, which also activates smooth membranes to bind polysomes, gives a curve of similar shape [1, 3]. It is, however, premature to draw any conclusions until the composition of the complex mixture comprising the extracts is analysed. It is noteworthy that male or female smooth membranes, which will not interact with either male or female polysomes separately, will combine with a mixture of male plus female polysomes. This mixture presumably contains, or replaces, all the hormone requirement for the formation of complexes of smooth membranes with polysomes.

In fig. 3 it is clearly demonstrated that degranulated rough membranes will bind polysomes from the same, but not the opposite sex. Binding of polysomes to degranulated membranes of the opposite sex does however take place if testosterone is added with the female and oestradiol with the male polysomes [7].

A summary of the requirements for membrane polysome interactions is given in table 1.

## 4. General conclusions

It is clear that the post microsomal supernatant does not contain any material which promotes the binding of polysomes to membranes. Male post microsomal supernatant will not replace testosterone for the binding of female polysomes to male degranulated membranes nor will female post-microsomal supernatant replace the oestradiol requirement for the interaction of male polysomes with female degranulated membranes. Furthermore, binding of polysomes to membranes also occurs in the presence of appropriate steroids in the absence of added supernatant.

Male and female polysomes contain a material, or materials, which will replace testosterone and oestradiol, respectively. Rough reticulum contains substances – not necessarily identical to those in the polysomes – which replace both testosterone and oestradiol. The nature of the active compounds is unknown and we shall refer to those from male and female polysomes as T and O substance, respectively. Experiments are planned to analyse the extracts to establish their exact chemical nature.

It is clear that male smooth membranes and polysomes are deficient in O substance and if this, or oestradiol, is added the polysomes will bind to the membranes. Female membranes and polysomes are similarly deficient in T substance. The level of O substance could control membrane–polysome interactions in the male and that of T substance similar interactions in the female.

It is possible that both hormones are actually required for interactions between membranes and polysomes to occur, with one of the hormones always present in the polysomes. There are, however, other possible explanations of our data and this problem can only be resolved by further work.

The identity of T and O substances is unknown although testosterone and oestradiol, respectively, can replace them in all systems and with identical specificity and selectivity. The possible importance of these naturally occurring activators of polysome binding to membranes in the control of the patterns of protein biosynthesis is very obvious and merits further detailed studies. We do not yet know whether there is any preferential binding of polysomes containing particular messengers or nascent proteins to the membranes. The experiments were not designed to detect possible selectivity in the binding process.

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