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ESTRADIOL BINDING IN THE CYTOSOL AND NUCLEAR PELLET OF ELECTROLYTICALLY TREATED RAT UTERI

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Summary

Intact mature rat uteri positioned in saline buffer at 0-2°C between platinum electrodes operating at a voltage in the range 1-4 VDC, with periodic reversal of polarity, are conditioned so that the cytosols obtained from these uteri lose 40% of specific and 32% of non-specific estradiol binding capacity and 90% of the dissolved protein. By contrast, estradiol bound in the nuclear pellet following incubation of the uterus in solution of the hormone shows a 50% increase in quantity if the uterus was electrolytically pretreated. The results are regarded as reflecting the removal from intact tissue by electrolytic treatment of a substance that inhibits and controls the translocation of estradiol receptor from the cytoplasm to the nucleus.

Evidence has been presented for the existence of a dialysable substance in the cytosol from rat uteri tissue that is believed to inhibit the translocation of estrogen receptor to the nucleus (1,2). Similar evidence has been reported with regard to mammary glands of mice (3). The results of the present study reveal that electrolytic treatment of intact rat uterine tissue prior to incubating the tissue in solution of estradiol at low or high temperatures results in a highly increased accumulation of the hormone in the nuclear pellet. This effect is thought to be due to the removal of translocation inhibitor from the cytoplasm of the intact tissue by the electrolytic treatment.

The phenomena of inhibition and activation of receptor translocation to the nucleus are of great interest because they may represent a key level of control of steroid hormone action. The reported study helps to expand the view on the translocation process.

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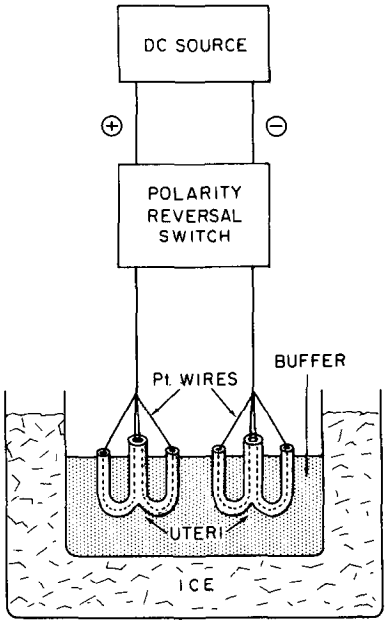

Fig. 1. Schematic of experimental arrangement for the electrolytic treatment of rat uterine tissue. Dashed line shows passage of Pt wire inside the uterus. The DC source is a Heathkit Regulated Power Supply Model IP-28. The polarity reversing switch is assembled from a latching relay and a timer equipped micro-switch.

Materials and Methods

Uteri from mature Sprague Dawley rats were flushed with ice cold saline and lengths of 29 gauge Pt wire were threaded through the horns. Two Pt supported uteri were positioned one cm apart in a 10 ml beaker, packed in ice (Fig. 1), containing 9 ml PBS (0.01M phosphate buffer, pH 7.4, with 9.0 g NaCl, 0.1 sodium azide and 1.0 g gelatin per liter). Depth of immersion was adjusted so as not to expose bare wire to bulk electrolyte. The Pt wires were connected to a DC source via a switch which reversed polarity every 20 seconds. The purpose in polarity reversal is to reduce the generation of concentration gradients, such as pH gradients, and to minimize the production of possibly bioactive substances at the electrodes. Uteri were treated at different voltages up to 4 V and for different lengths of time at 4 V. After treatment the horns were slit open and the tissue thoroughly rinsed with ice cold saline. Two procedures were followed.

In intact tissue incubation procedure the uteri were incubated in 8 nM (^{3}H)-estradiol (6,7^{3}H) in PBS, 2 ml/uterus, for 24 hours at 4°C or for 7 hours at 4°C then 1.5 hours at 22°C followed by a final 15.5 hours at 4°C. After incubation the uteri were rinsed with ice cold saline, shattered to powder in a steel mortar chilled with LN_2, and the powder homogenized on ice in a glass-on-glass unit using 2 ml PBS per uterus. The homogenate was centrifuged at 1000 x g, the pellet washed twice with ice cold saline with centrifugation after each washing, and finally extracted with ethanol, 2 ml/uterus, at room temperature for 1 hour. The label in the extract was determined by scintillation counting.

Table 1

Additives	Fmoles/uterus		Fmoles/mg protein	
	Untreated uteri	Treated uteri	Untreated uteri	Treated uteri
—	5300	3413	589	4266
E	2363 (2937)	1741 (1672)	262 (326)	2176 (2090)
Tam	2726 (2574)	1833 (1580)	303 (286)	2291 (1975)
DES	2491 (2809)	1545 (1868)	276 (312)	1931 (2335)

Binding of (^{3}H)-estradiol in cytosol from untreated and from electrolytically treated rat uteri in the presence and absence of excess unlabeled estradiol (E), Tamoxifen (Tam), or Diethylstibestrol (DES). The numbers in parenthesis give the arithmetic difference between binding in the presence and absence of the respective additives and provide an estimate of specific estrogen binding by receptors. (Details in text.)

The second procedure was concerned with estradiol binding in high speed cytosol. A homogenate was prepared as described above but from non-incubated uteri and centrifuged for 1 hour at 105,000 x g. The high speed cytosol was treated with a DCC pellet (0.025 g charcoal per 1 ml cytosol) for 20 minutes on ice to remove free or loosely bound endogenous estradiol. The DCC pellet was derived from a dispersion composed of 5 g Norit A charcoal in 100 ml PBS (no gelatin) containing 0.5 g dissolved Dextran T70 (Pharmacia). Each test solution had a total volume of 1 ml and in addition to PBS and 0.2 ml cytosol comprised 9.5 picomoles of (^{3}H)-estradiol and either no additives or one of the following: 0.95 nanomoles of unlabeled estradiol (E), 0.95 nanomoles of diethylstilbestrol (DES), or 95 nanomoles of Tamoxifen (Tam) (ICI America). The stock solution of E and DES was prepared with pure ethanol, while that of Tam was prepared with the mixture 80% EtOH - 20% DMF. Each additive was introduced to the test solution as 10 µl aliquots of the stock solution. Test solutions without additives contained 10 µl ethanol blank. Incubation was 16 hours at 4°C. The incubated mixtures were treated with 0.1 ml aliquot of the DCC dispersion on ice for 20 minutes, centrifuged, and the label in the supernatant determined by scintillation counting. Protein content in the cytosols was determined by the Lowry method (4).

Results

Cytosol

The results in Table 1 compare (^{3}H)-estradiol binding in cytosols from untreated and from electrolytically treated uteri. Electrolytic treatment was 30 minutes at 4 V. The protein content found in cytosols from the untreated and from the electrolytically treated uteri was 4.5 mg and 0.4 mg per ml respectively.

Incubation of 0.2 ml aliquots of cytosol with 9.5 nM (^{3}H)-estradiol is sufficient to substantially saturate the receptor sites with the labeled hormone in the absence of competing ligands (5). To estimate specific binding,

parallel incubations were conducted with a hundred-fold excess of either E or DES (5), or a ten thousand-fold excess of Tam relative to (^{3}H)-estradiol. The intent in using the much larger excess of Tam is to compensate for its significantly smaller association constant with the receptor compared to that of estradiol (6,7). The results show that at these excess quantities the three ligands have comparable effectiveness in competing with (^{3}H)-estradiol for specific binding sites.

Nuclear Pellets

After completion of ethanol extractions all nuclear pellets were dried for 4 days at ambient temperature to a constant weight. There was no difference in the average weight of the pellets, 40.1 ± 4.6 mg/uterus, whether these were derived from untreated or from electrolytically treated uteri.

Fig. 2 shows the effect of duration of electrolytic treatment of the uteri at 4 V, prior to incubation with (^{3}H)-estradiol, on the quantity of hormone extracted from the nuclear pellet. The separation between the two curves shows the effect of exposing the system to 22°C (8,9), and thereby thermally activating the receptor-hormone complex for translocation to the nucleus.

Fig. 3 shows the effect of voltage at which electrolytic pretreatment was conducted on the quantity of hormone extracted from the nuclear pellet. Treatment was for 20 minutes at each of the selected voltages. The uteri in this experiment were incubated with (^{3}H)-estradiol for 24 hours at 4°C only.

As seen, increase in voltage up to 2 V results in increase in the quantity of hormone extracted from the pellet. Beyond 2 V this quantity remains at a plateau. Comparing the result for 20 minutes treatment of 4 V in Fig. 3 with the result in Fig. 2 for the same conditions, the value in Fig. 2 is seen to be about 20% lower. This difference arises because different batches of rats were used in each of these experiments. Each experimental point in both experiments was determined on a pool of four uteri.

In the electrolytic treatment the current passing through the system was 1 mA at 1 V, 1 mA at 1.5 V, 3-5 mA at 2 V, 7-13 mA at 3 V, and 15-22 mA at 4 V.

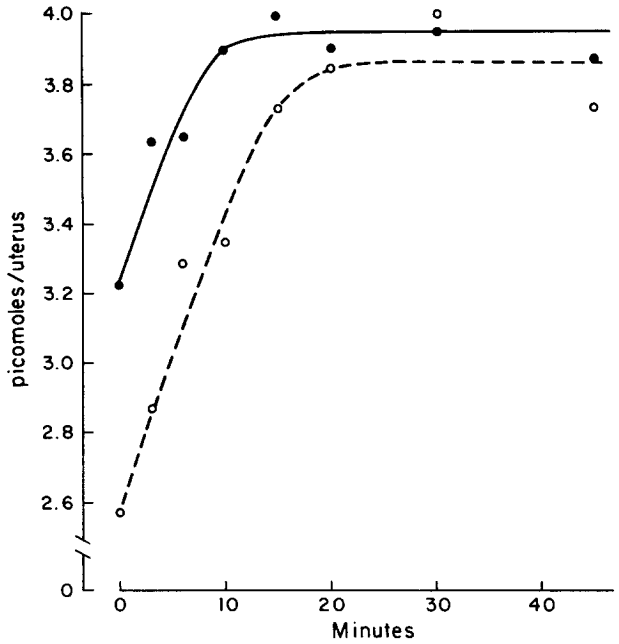

Fig. 2. Effect of duration of electrolytic treatment of uteri at 4 V, prior to incubation with (^{3}H)-estradiol, on the quantity of hormone extracted from the nuclear pellet. (●) Uteri incubated at 22°C for 90 minutes in addition to 22.5 hours at 4°C. (◎) Uteri incubated 24 hours at 4°C only. (Details in text.)

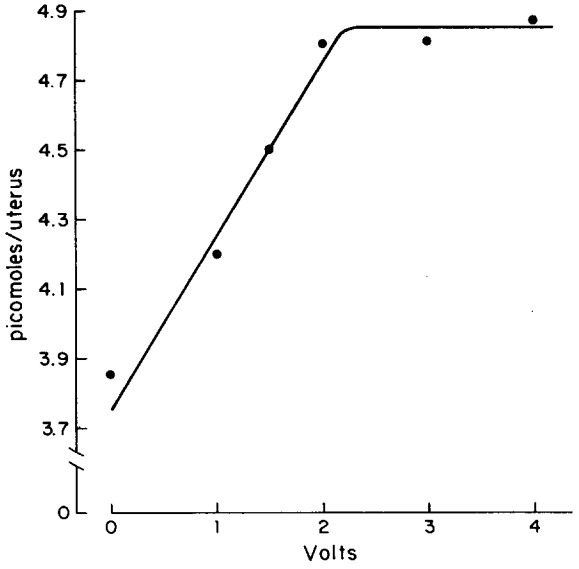

Fig. 3. Effect of voltage in electrolytic treatment of uteri prior to incubation with (^{3}H)-estradiol on the quantity of hormone extracted from the nuclear pellet. Electrolytic treatment conducted for 20 minutes. Uteri were incubated for 24 hours at 4°C only. (Details in text.)

At the first instant after polarity reversal the higher current flow of the indicated range was noted and this declined withint 2 seconds to the lower value which remained stable for the remaining 18 seconds of the polarity reversal half cycle.

Electrolyte

Electrolyte (9 ml) in which 2 pairs of uteri were treated, one pair succeeding the other, for a total of 90 minutes at 4 V, showed no measurable increase in protein content. The pH of the electrolyte changed from 7.30 to 7.27 after use.

Substituting 0.4 ml of the "used" electrolyte for 0.4 ml of buffer in cytosol test solutions, such as those described earlier, had an effect neither on specific nor on non-specific hormone binding. Determinations were made in the presence and absence of excess Tam or DES and with cytosols from untreated and from electrolytically treated uteri.

Discussion

The central finding in this work is that electrolytic treatment of uterine tissue prior to incubation with estradiol results in a marked increase in hormone bound in the uterine pellet (Figs. 2 and 3). This increase overrides the effect of thermal activation of the receptor hormone complex for translocation to the nucleus. The source of this increase may be the electrolytic removal or electrolytic deactivation of an inhibitor to receptor translocation to the nucleus. One may hypothesize that the process of thermal activation of the receptor-estradiol complex for translocation is composed of dissociation of the inhibitor from the complex followed by transformation of the complex to a form which is affected by the inhibitor to a much lesser degree. Thermal activation is associated with the transformation of the receptor complex from a species sedimenting on sucrose gradient centrifugation at 4S to one sedimenting at 5S (8,9). One may consider then a model where the inhibitor associates more strongly with the 4S than with the 5S complex. In these terms, actual removal or deactivation of the inhibitor would then result, as is indeed observed

here, in a more extensive migration of receptor to the nucleus than that arising from thermal activation.

An alternative hypothesis is that electrolytic treatment alters the receptor itself and imparts to it higher binding capacity for the hormone. Such an increment in capacity cannot be caused by a possible electrolytic removal of endogenous estradiol from the tissue because, as reported here, admixtures of heavily used electrolyte to cytosol test solutions have shown no effect on labeled hormone binding by the receptors.

The cytosol of electrolytically treated uteri is depleted both of hormone binding capacity and of protein (Table 1). The reduction in binding capacity may reflect simply the spontaneous migration of the receptor to the nucleus upon the removal of inhibitor. Sato et al. (1,2) have already shown that receptor in dialysed cytosol can readily translocate to the nuclei in the absence of hormone. Alternatively it is possible that the receptor protein along with other soluble cytoplasmic proteins is electrodeposited on insoluble parts of the cell other than the chromatin and in this manner it is included in the pellet. However, the fact that protein depletion is greater than 90% while specific binding loss is less than 40% (Table 1) detracts from this possibility. In fact, it appears from these results that neither specific nor non-specific hormone binding in the cytosol is proportionately associated with the overall quantity of protein.

Increased accumulation of estradiol in the pellet is induced by electrolytic treatment of uteri at voltages well below and well above the threshold voltage for water electrolysis ($\sim$ 1.5 V including over-voltage). Therefore, oxygen-hydrogen evolution or transient pH changes in the vicinity of the tissue cannot be the cause of the phenomenon. Similarly, the phenomenon cannot be related, at least directly, to the magnitude of the current passing through the system because in the range from 0 to 2 volts the plot in Fig. 3 is linear, but the current in the same voltage range is not.

The following points of data support the view that the additional hormone found in the pellets of treated uteri is specifically bound. Firstly, the loss of specific binding in the cytosol of treated uteri is matched in range by the gain in the quantity of hormone found in the pellet, 1.1 and 1.3 picomoles/uterus respectively. The 1.1 value derives from the difference in the average of specific binding between treated and untreated uteri in Table 1; the 1.3 value derives from the data in Fig. 2. Secondly, the establishment of the hormone binding plateau (Fig.2) can mean that the binding is saturable.

Acknowledgement

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