3H-PROGESTERONE BINDING IN THE OVIDUCT OF THE LITTLE SKATE RAJA ERINACEA

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The skate Raja erinacea is an oviparous species, with a reproductive period characterized by short cycles, in which eggs are produced in pairs every 5-7 days (Richards et al., Bull. Bingham Oceanogr. Collect. 18:1-67,1963). A follicular phase, characterized by high levels of progesterone and testosterone, is followed by a short luteal phase. Plasma progesterone and testosterone levels decrease sharply after ovulation and plasma estradiol levels are also reduced (Koob et al., Biol. Reprod., 35:267-275, 1986). As in all other vertebrates, the reproductive tract is under the control of ovarian hormones. Once fully developed, cyclic changes occur according to the reproductive cycle. Estradiol is implicated in the development and mantainance of the reproductive tract, as suggested by the correlation between plasma estradiol levels and the shell gland size (Koob et al., Biol. Reprod., 35:255-267, 1986). The role of progesterone in supporting the oviduct activity is less clear. Progesterone treatment accelerates oviposition and reduces the egg-capsule retention time in the reproductive tract, from 3-4 days to less than one day (Koob and Callard, The Bull., Mt. Desert Isl. Biol. Lab., 25:138-139, 1985). The site of progesterone action is still unknown. The effect may be either indirect, through the hypophysis, or direct, through depletion of oviduct steroid receptors. In order to elucidate the mechanism of progesterone action on the oviduct regulation, we investigated the presence of ^{3}H-progesterone binding in the oviduct of the skate Raja erinacea. Here we report some preliminary data on the characteristics of the progesterone receptor.

Skates were caught off the coast of Maine and maintained in flow-through circulating sea-water tanks at ambient water temperatures during July and August, 1993. Animals were sacrified by pithing. The oviducts were removed and rinsed in saline buffer for elasmobranchs (Foster et al., Comp. Biochem. Physiol., 42A:3-12, 1972) at 4 °C, to wash out the blood. Cytosol and nuclear extract were obtained by ultracentrifugation as described in Reese and Callard (Gen. and Comp. Endocr., 84:170-181, 1991). Aliquots (200 μl) of cytosol and nuclear extract were incubated with increasing concentrations of ^{3}H-progesterone (from 0.6 to 80 nM) with or without 400 fold cold progesterone. Free steroid was removed by Sephadex LH-20 chromatography. Counts obtained by incubating the sample (cytosol or nucleare extract) with ^{3}H-progesterone alone were the total binding (T), whereas counts obtained by incubating the sample (cytosol or nuclear extract) with ^{3}H-progesterone plus cold progesterone were the non specifc binding (NS). Specific binding (S) was obtained by subtracting non specific binding from total binding. Saturation and Scatchard analysis for the nuclear extract are shown in Fig. 1 and 2 respectively. The binding saturated between 40 and 80 nM ^{3}H-progesterone. The Kd was 2.9 x 10^{-8} M, with a B_{max} of 2.1 x 10^{-10} M. Specificity of ^{3}H-progesterone binding was studied by competition analysis. Aliquots (200 μl) of cytosol and nuclear extract were incubated with ^{3}H-progesterone in presence or in absence of 10- 100- 1000-fold non labeled steroids. Data reveal that the binding is specific for progesterone. The order was:

progesterone> Δ 5 pregnenolone > R5020 > deoxycorticosterone > testosterone > estradiol-17β.

In conclusion we report for the first time the presence of a progesterone receptor in the oviduct of an elasmobranch, the skate Raja erinacea. The progesterone receptor characteristics (Kd, saturation, specificity) are in good agreement with those of other vertebrates (Callard and Callard, in: Hormones and Reproduction in Fishes, Amphibians and Reptiles, 355-384, 1987. Ed. by D.O. Norris and R.E. Jones, Plenum Press, New York and London).

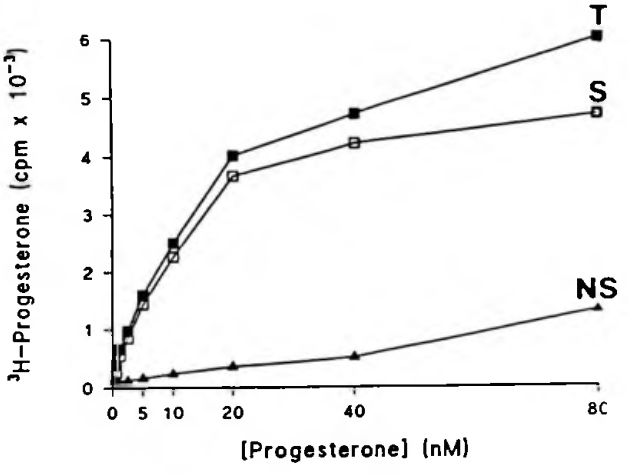

Fig.1 Saturation analysis of ^{3}H-progesterone binding in the nuclear extract of Raja erinacea oviduct. (T= total binding; S= specific binding; NS= non specific binding)

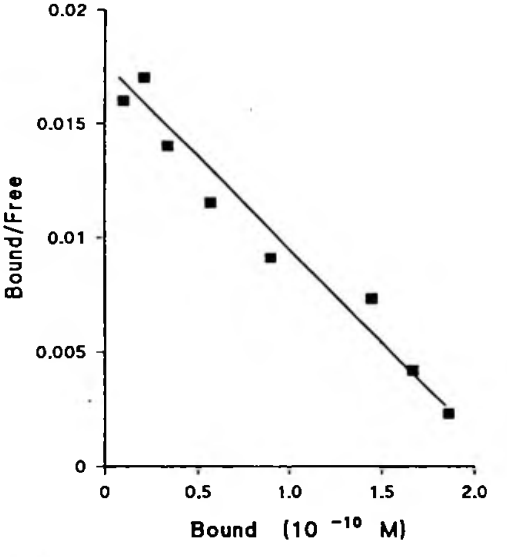

Fig.2 Scatchard analysis of ^{3}H-progesterone binding in the nuclear extract of Raja erinacea oviduct

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