INHIBITION OF OESTROUS BEHAVIOUR IN EWES BY PASSIVE IMMUNIZATION AGAINST OESTRADIOL-17 β

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The biological activity of a steroid can be readily neutralized by high-affinity antibodies directed against the steroid, thus providing a useful method of studying its effects. Biological neutralization with antibodies to low molecular weight hormones can be produced either by active immunization against an hormonal hapten (Scaramuzzi, Caldwell, Tillson & Thorneycroft, 1970), or by the passive transfer of antibodies to the hormone (Vande Wiele, Ferin, Raziano, Dyrenfurth & Mikhail, 1970). The widespread use of these methods has not been possible in the past because of difficulties in generating antisera to steroid haptens and the relatively small quantities of antisera produced. The availability of reliable quick methods for the production of antisera to hormones of low molecular weight (Vaitukaitis, Robbins, Nieschlag & Ross, 1971) has been reflected by the increased application of these methods to biological studies (Cowchok & co-authors, 1973; Hillier, Cole, Groom, Boyns & Cameron, 1973; Nieschlag, Usadel, Schwedes, Kley, Schöffling & Kruskemper, 1973; Sundaram, Tsong, Hood & Brinson, 1973; Thorneycroft, 1973).

Active immunization results in long-term neutralization of hormonal activity. Neutralization by the passive transfer of antisera would be preferable when, as during the oestrous cycle, the hormonal environment is changing rapidly. In the present report, antisera specific to oestradiol-17 β have been used to neutralize the ability of oestradiol benzoate (ODB: 25 μ g) to induce oestrus in the ovariectomized ewe primed with progesterone.

Antiserum to oestradiol-17 β was produced in large quantities by immunizing six wethers against a conjugate of oestradiol-6-bovine serum albumen (BSA) as described by Scaramuzzi, Corker, Young & Baird (1974). Fifty-four separate antisera (nine from each of the six animals) were pooled to give approximately 6 litres of anti-oestradiol serum which was then characterized (Scaramuzzi *et al.*, 1974) and used in the tests described.

Sixteen ewes, ovariectomized at least 2 months earlier, were used in a series of five tests conducted at monthly intervals during the 1973/74 breeding season. Before each test, all ewes were primed with intramuscular injections of 10 mg progesterone daily for 12 days (Robinson, 1959) and, 48 hr after the last injection, 25 μ g ODB were administered to induce oestrous behaviour. The anti-oestradiol serum or non-immune control serum was given by subcutaneous injection 3 hr before ODB. In some tests, the antiserum was administered simultaneously with the ODB. For the following 72 hr, the ewes were placed in

TEXT-FIG. 1. Characteristics of pooled antiserum to oestradiol-6-bovine serum albumen. (a) The titre of the pooled antiserum; (b) inhibition curves for oestradiol-17 β , oestradiol benzoate and oestrone.

TEXT-FIG. 2. The percentage binding of [6,7-³H]oestradiol-17 β to three dilutions of plasma from ewes passively immunized with different volumes of antiserum to oestradiol-6-bovine serum albumen (two to six ewes) or to two dilutions of plasma from ewes previously injected with 10 ml non-immune serum (four ewes). The points and vertical bars represent the Means $\pm$ S.E.M.

a field with a raddled ram and were examined twice daily for evidence of oestrous behaviour. Antiserum titres were measured in samples of jugular venous plasma taken immediately before, and at 1, 2 and 3 days after passive immunization. The procedures used to determine the titre and the specificity of the pooled antiserum have been described in detail by Scaramuzzi *et al.* (1974). The specificity and titre of the pooled antiserum used to produce passive immunization of the ewes are illustrated in Text-fig. 1. The titre of an antiserum is defined as that dilution which binds 50% of the added tracer (Thorneycroft, Tillson, Abraham, Scaramuzzi & Caldwell, 1970) and was 1/14,000 for the pooled antiserum. Cross-reactions (relative to oestradiol-17 β) for oestrone, oestradiol benzoate and oestradiol-17 α (not illustrated) were 7.5%, 77.8% and 3.0% respectively at 50% displacement (Text-fig. 1). The titres of antiserum in ewes passively immunized with pooled antiserum are shown in Text-fig. 2. The binding at two dilutions of plasma from ewes immunized with non-immune serum was negligible ($5.4 \pm 1.1\%$ at 1/10, and $2.2 \pm 0.2\%$ at 1/100). The quantity of [6,7- ^3H] oestradiol-17 β which was bound showed a linear increase with the increasing amounts of anti-oestradiol serum injected (Text-fig. 2).

The antiserum titre remained unchanged for up to 72 hr after passive immunization (Table 1) and, in some cases, low but detectable antiserum titres were present 28 days after passive immunization.

Table 1. Percentage binding of [6,7- ^3H]oestradiol-17 β to dilutions of plasma at various intervals after subcutaneous injection of 10 ml antiserum to oestradiol-6-BSA or 10 ml non-immune serum

Serum treatment	No. of ewes	Time after injection (days)	Dilution of plasma				
			1/10	1/50	1/100	1/500	1/1000
Non-immune serum	4	1	5.4 ± 1.0	2.8 ± 0.2	2.2 ± 0.2	2.2 ± 0.1	1.7 ± 0.1
Antiserum	6	1	74.5 ± 2.6	37.2 ± 4.5	22.2 ± 2.9	6.3 ± 0.7	4.3 ± 0.4
Antiserum	6	2	81.0 ± 1.6	39.6 ± 2.5	26.7 ± 1.1	6.6 ± 0.2	4.4 ± 0.2
Antiserum	6	3	73.7 ± 2.7	44.3 ± 3.5	23.8 ± 2.1	6.5 ± 0.5	4.3 ± 0.4
Antiserum	4	28	46.0 ± 2.9	12.0 ± 1.1	7.0 ± 0.9	2.0 ± 0.0	1.5 ± 0.2

Values are expressed as means $\pm$ S.E.M.

The proportions of ewes showing oestrous behaviour in each control group was almost 100% and the small difference was not significant (11/11 untreated controls versus 7/8 controls treated with 10 ml non-immune serum). Treatment with 10 ml anti-oestradiol serum 3 hr before ODB depressed the oestrous response (1/13 versus 7/8). When 10 ml antiserum were injected simultaneously with ODB, the blocking of oestrous behaviour was less effective and a smaller difference was observed (3/6 versus 7/8). Likewise 5 ml antisera injected before ODB did not inhibit oestrous behaviour very effectively (4/8 versus 7/8).

The present data show that large volumes of specific antisera with high titres against oestradiol-6-BSA can be readily produced by immunizing sheep. There can be little doubt that similar results could be obtained with other protein-steroid conjugates (Scaramuzzi *et al.*, 1974). The specificity of the pooled antisera against oestradiol-6-BSA has been determined *in vitro* at a dilution of

1/20,000 and these data show that the antiserum has a high degree of specificity for oestradiol-17 β . The specificity of the antiserum *in vivo* and at much higher concentrations of antibodies was not determined.

The ovariectomized ewe primed with progesterone is a well-established model for assessing the effect of oestrogen and 25 μ g ODB will reliably induce oestrus in 90% or more of such sheep (Robinson, 1959). In the present experiments, 18/19 of the progesterone-primed ewes given ODB without antiserum showed oestrous behaviour. The ability of antisera to oestradiol-6-BSA to achieve partial neutralization of the oestrus-inducing activity of 25 μ g ODB given at the same time (Table 1) shows that the antiserum is quickly absorbed following subcutaneous injection. No change was seen in the oestradiol-binding capacity of plasma taken from ewes up to 72 hr after passive immunization, indicating that antibodies against oestradiol persist in the circulation for at least 3 days (Table 1).

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