Evidence for direct neural control of ovarian steroidogenesis in rats

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Summary. Electrical stimulation of the superior ovarian nerve of intact anaesthetized dioestrous rats for 30 min reduced ovarian progesterone levels, even when papaverine and propranolol were also given. The administration of phentolamine (an alpha receptor antagonist) before stimulation reversed this effect. The results suggest that a neural control of ovarian steroidogenesis may be either excitatory through the stimulation of beta receptors of inhibitory through the stimulation of alpha receptors.

Introduction

Most endocrine glands are innervated by the autonomic nervous system and the role of these nerves in the control of hormone production has attracted considerable interest (Ganong, 1974; Melander, Ericson & Sundler, 1974; Smith & Porte, 1976). The adrenergic receptors, particularly the beta receptors, have been implicated as mediators of many of the effects of the autonomic nervous system (Melander, Ranklev, Sundler & Westgren, 1975; Brown, Hurwitz, Woodward & Aurbach, 1977; Lundquist & Ericson, 1978). The sympathetic nerves to the ovary are known to innervate smooth muscle cells of the follicle, the ovarian vasculature and steroid-producing cells (Burden, 1978; Mohsin & Pennefather, 1979). The research attempting to define a functional role for the ovarian sympathetic fibres has mostly focussed on the control of ovarian smooth muscle contraction and its contribution to ovulation (Marshall, 1973; Weiner, Wright & Wallach, 1977). The possible role of this innervation in the control of ovarian steroidogenesis has received relatively little attention. Of particular interest are reports showing the apparent synapses of axons, which appear to be adrenergic, with steroid-producing cells in ovaries of the chicken (Dahl, 1970), cat (Jacobowitz & Wallach, 1967), mouse (Unsicker, 1974) and rat (Burden, 1972). Lawrence & Burden (1980) have used fluorescence histochemical techniques to trace the adrenergic innervation to the ovary: one route is via the ovarian plexus and the fibres terminate primarily on blood vessels while the second route (superior ovarian nerve) follows the suspensory ligament and fibres terminate on blood vessels and the steroid-producing cells of the interstitial gland. Lawrence & Burden (1980) concluded that this pattern of innervation provided a potential for a direct nerve modulation of steroidogenesis in the interstitial gland. Burden & Lawrence (1977) had earlier shown that ovarian denervation resulted in a decrease in Δ4-3β-hydroxysteroid dehydrogenase activity in both the interstitial gland and corpus luteum in the pregnant rat. This reduced activity was interpreted as a decreased capacity of denervated ovaries to synthesize progesterone. Capps, Lawrence & Burden (1978) showed that stimulation of the ovarian plexus nerve in hypophysectomized rats induced morphological changes in the interstitial gland characteristic of active steroidogenic cells. Moreover, plasma progesterone concentrations were decreased on the first day of metoestrus that occurred in ovariectomized female rats bearing subcutaneous ovarian grafts.
(Chihal, Weitsen, Stone & Peppler, 1976). These responses of interstitial gland cells to
denervation and stimulation provided morphological evidence for a functional role for the
adrenergic nerves to the ovarian compartment.

Adrenergic receptor agonists cause a significant increase in the production of cyclic AMP
and progesterone in ovarian tissue in vitro (Condon & Black, 1976; Jordan, Caffrey &
Niswender, 1978; Ratner, Sanborn & Weiss, 1980). These effects can be blocked with beta
receptor antagonists and are consistent with the suggestion that sympathetic nerves to the ovary
might enhance steroidogenesis by means of a beta adrenergic mechanism. This report tests the
hypothesis that the sympathetic fibres of the superior ovarian nerve may alter ovarian
steroidogenesis.

Methods and Materials

Female Sprague-Dawley rats (200–300 g) were housed in temperature- (24 ± 1°C) and
light-(14 h light/day; 06:00–20:00 h) controlled quarters and fed rat chow and water ad
libitum. Vaginal smears were taken each day and only those animals that showed 3 consecutive
4-day cycles were used. All experiments were performed on the second day of dioestrus between
09:00 and 13:00 h. Rats were anaesthetized with pentobarbitone sodium (35 mg/kg) and a
midline abdominal incision provided access to the ovaries. The suspensory ligament containing
the superior ovarian nerve was isolated from surrounding tissue, ligated and cut cranial to the
ovary. A bipolar silver wire electrode was placed on the distal isolated superior ligament close to
the ovary and lowered into a pool of mineral oil. The nerve was stimulated 1 min on and 1 min
off with constant current (100–400 μA) at 20–30 Hz for a period of 30 min. This stimulus was
considered to be supramaximal and should have activated all the fibres within the superior
ovarian nerve that go to the ovary on the same side as the nerve. The ovary on the opposite side
was not stimulated. At the end of the stimulation period, the stimulated and the unstimulated
ovary of each rat were removed, weighed and frozen.

Both ovaries were extracted and assayed for progesterone by the radioimmunoassay
described by Orczyk, Hichens, Arth & Behrman (1978). The standards were made from
progesterone purchased from Sigma Chemical. The progesterone antiserum was from the P-1
pool of Dr H. R. Behrman and had percentage cross-reactivity of 0-1% for 20α-dihydro-
progesterone and <0-02% for oestradiol-17β, oestrone and dihydrotestosterone. The sensitivity
was 50 pg/ml serum and the extraction efficiency was 75–97%. The concentration of
progesterone was calculated per mg tissue.

The significance of differences between control and stimulated ovaries was assessed by a
paired t test; a P value of 0.05 was considered significant. All drugs were injected into the
jugular vein 20–30 min before nerve stimulation in volumes of <1 ml. These drugs included
phenotolamine (Regitine hydrochloride) from Ciba at a dose of 6–8 mg/kg, papaverine
hydrochloride from Lilly at 8 mg/kg, and Dl-propranolol hydrochloride from Ayerst at 2–6
mg/kg. Animals receiving no drug were injected with a comparable volume of saline (9 g
NaCl/l).

Results

As shown in Table 1, three of the treatments caused a decrease of progesterone content in the
stimulated ovary as compared to the control ovary taken from the same rat. An increase in
progesterone concentration was obtained only when the alpha receptor antagonist (phen-
tolamine) was administered.
**Table 1.** Effect of nerve stimulation alone and in combination with various drug treatments on rat ovarian progesterone

<table>
<thead>
<tr>
<th></th>
<th>Exp. A (stimulation only)</th>
<th>Exp. B (stimulation + phentolamine)</th>
<th>Exp. C (stimulation + propranolol)</th>
<th>Exp. D (stimulation + papaverine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Control ovary</td>
<td>48.3 ± 4.3</td>
<td>40.1 ± 1.4</td>
<td>47.2 ± 4.7</td>
<td>49.1 ± 4.6</td>
</tr>
<tr>
<td>Stimulated ovary</td>
<td>32.7 ± 3.6*</td>
<td>55.4 ± 3.2*</td>
<td>29.6 ± 4.2*</td>
<td>38.4 ± 4.1*</td>
</tr>
<tr>
<td>% Change</td>
<td>−32.3</td>
<td>+38.2</td>
<td>−37.2</td>
<td>−21.6</td>
</tr>
</tbody>
</table>

Values are mean ± s.e.m.
* Significantly different from control, *P < 0.05 (t test).

**Discussion**

When all the fibres within the superior ovarian nerve are stimulated, as in the present experiments, the sympathetic fibres to different cells in the ovary are all simultaneously activated and any changes in progesterone will reflect a combination of different influences on ovarian steroidogenesis. There may be an indirect effect due to blood flow changes combined with a direct influence of the nervous activity on steroidogenesis. Changes in blood flow alone might alter the removal rate of the progesterone and thus could be falsely interpreted as a change in progesterone synthesis. A substantial decrease in blood flow induced by an augmented sympathetic nerve activity could deprive the ovarian cells of the necessary nutrients to sustain progesterone synthesis. Because of these possibilities, the various adrenergic receptors were selectively blocked to determine whether the sympathetic nerves have any direct effect on progesterone production in the ovary. The working hypothesis for these experiments, derived from our previous in-vitro work (Ratner et al., 1980), was that any direct excitatory effect on steroidogenesis would be mediated by beta adrenergic receptors during sympathetic nerve stimulation. In addition, there may exist a direct inhibitory influence on steroidogenesis which is mediated through alpha receptors.

The results of Exps A and B suggest that some sympathetic nerves to the ovary produce a decrease in progesterone which is mediated by alpha receptors. It appears that this effect overrides an additional, simultaneous beta excitatory drive which could act to increase steroidogenesis (Exp. B). One interpretation of these results is that an extensive constriction of the ovarian vasculature during the nerve stimulation when no alpha antagonist is present (Exp. A) reduces the blood flow to such an extent that progesterone production is compromised in spite of the existing drive to increase steroid production. We have observed a marked decrease in ovarian blood flow during ovarian nerve stimulation in experiments in which the ovarian venous blood was being continuously collected (unpublished data). A second possibility is that there exists a direct alpha receptor-mediated inhibitory effect on steroid-producing cells, which either alone or in combination with a reduced blood flow would produce a reduction in steroid output. An alpha receptor antagonist would block both the vascular response and any direct effect on steroid cells and thereby allow full expression of the excitatory influence of the nerve stimulation. This is what we believe occurred in the phentolamine-treated animals (Exp. B). A direct alpha receptor-mediated effect on hormone production has been observed in other endocrine glands (Melander, Sundler & Ingbar, 1973; Brown, Hurwitz & Aurbach, 1978; Rabino-vitch, Cersi & Sharp, 1978). A particularly good example is the pancreas in which the sympathetic nerves have an inhibitory effect on insulin release that operates through alpha receptors (Jarhult & Holst, 1979).
The results of Exp. D with papaverine were also consistent with the above interpretation. Since the alpha receptors were still functioning in Exp. D, but were unable to produce vascular constriction, a direct alpha receptor-mediated decrease in steroidogenesis appears to be strong enough to override the simultaneous excitatory influence by the nerves. Papaverine is a phosphodiesterase inhibitor often used to augment cyclic AMP-mediated responses. Our previous in-vitro studies suggested that a beta receptor stimulation of progesterone synthesis involves the activation of the adenylate cyclase–cyclic AMP system (Ratner et al., 1980). This would confound the present results since the papaverine given to block the vascular response might also augment the increase in tissue progesterone levels produced by the stimulation. If this interpretation is correct, the decrease in tissue progesterone in the papaverine-treated rats is less than it should have been, further suggesting the possibility of a direct alpha-mediated decrease in tissue progesterone levels.

When propranolol, a beta receptor antagonist, was administered (Exp. C), the response was not significantly different from that observed in Exp. A when no drug treatment was involved. If the competitive effects of a simultaneous drive to both decrease and increase steroidogenesis were strictly additive, we would expect to see a further decrease after removal of the beta excitatory drive. This is difficult to explain. One possibility is that blood flow decreases due to the vascular constriction and may not only compromise ongoing progesterone production but also prevent the actual expression of any increased excitatory phenomenon. The blocking of the excitatory mechanism with a beta receptor antagonist would have little effect because the expression of the excitation was not present before the blockade.

In these experiments, we have measured changes in the concentrations of ovarian progesterone. It is possible that the observed effects could be explained by changes in the release of progesterone from the tissue and not by changes in steroidogenesis. However, this seems unlikely since ovarian steroid production is considered to by synonymous with release (Baird, 1977).

We conclude that stimulation of ovarian sympathetic fibres may influence ovarian progesterone production directly. These direct effects may be excitatory through the activation of beta receptors or inhibitory through the stimulation of alpha receptors. In addition, it is suggested that the changes in blood flow induced by sympathetic nerve activity can indirectly control the ovarian steroid levels and significantly alter direct receptor-mediated effects. Even though the entire ovarian distribution of sympathetic nerves was simultaneously stimulated in these experiments, under normal physiological conditions, the central nervous system might selectively activate any one of the different pathways. The experiments done by Kawakami, Kubo, Uemura, Nagase & Hayashi (1981) support this possibility: specific areas in the brain of hypophysectomized and adrenalectomized rats were stimulated and resulted in change in ovarian steroidogenesis without changes in ovarian blood flow. Physiologically, the sympathetic innervation may play a permissive role, modulating the effect of trophic hormones, particularly LH, on ovarian steroidogenesis. It would also be more likely that these direct neural effects are primarily on the interstitial gland cells because they appear to have an extensive sympathetic innervation, but this does not exclude the possibility of direct effects on other steroidogenic compartments in the ovary.

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References


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