A role for alternative pathway catecholamines in the regulation of steroidogenesis in cow luteal cells

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Summary. Incubation of bovine luteal cells with the alternative pathway catecholamines octopamine, synephrine and deoxyadrenaline at concentrations of 10^{-6} to 10^{-3} M enhanced the production of progesterone ($P < 0.05$). Tryamine did not alter basal progesterone production ($P > 0.05$). Addition of noradrenaline and adrenaline at concentrations of 10^{-4} to 10^{-7} M significantly elevated the production of progesterone ($P < 0.05$). The steroidogenic response to noradrenaline and adrenaline was greater than that for octopamine, synephrine and deoxyadrenaline ($P < 0.05$). Response to both primary (10^{-6} M) and alternative (10^{-4} M) pathway catecholamines was inhibited by propranolol (10^{-5} M, $P < 0.05$) but not phentolamine (10^{-5} M, $P > 0.05$). These results demonstrate that octopamine, synephrine and deoxyadrenaline can affect steroidogenesis by bovine luteal cells, and their action is mediated by β -adrenergic receptors.

Introduction

The primary catecholamines noradrenaline and adrenaline can regulate the synthesis *in vitro* of cAMP and progesterone by luteal tissue from a variety of species (cow: Condon & Black, 1976; Godkin, Black & Duby, 1977; Milvae, Alila & Hansel, 1983; rat: Harwood, Dufau & Catt, 1979; Ratner, Sanborn & Weiss, 1980; Norjavaara, Selstam & Ahren, 1982; rabbit: Birnbaumer, Yang, Hunzicker-Dunn, Brockaert & Duran, 1976; ewe: Jordan, Caffrey & Niswender, 1978). Noradrenaline and adrenaline can interact with both α - and β -adrenergic receptors although their stimulatory effects on luteal steroidogenesis are mediated by β -receptors, since their response can be inhibited by β -adrenergic antagonists but not by α -adrenergic antagonists (Condon & Black, 1976; Jordan *et al.*, 1978; Harwood, Richert, Dufau & Catt, 1980; Ratner *et al.*, 1980; Norjavaara *et al.* 1982). In contrast, nothing is known about the catecholamines derived through the alternative catecholamine pathway (Fig. 1) with respect to ovarian steroidogenesis. Octopamine and synephrine have been identified within the central nervous system and peripheral tissues in a number of vertebrate species (Kakimoto & Armstrong, 1962; Molinoff & Axelrod, 1969, 1972; Saavedra, 1974; Harmar & Horn, 1976). In peripheral tissues octopamine is associated with sympathetic nerve endings (Molinoff & Axelrod, 1969, 1972) where it can be taken-up, synthesized and stored (Fischer, Musacchio, Kopin & Axelrod, 1964; Kopin, Fischer, Musacchio & Horst, 1964; Kopin, Fischer, Musacchio, Horst & Weise, 1965; Baldessarini & Vogt, 1971). Octopamine accumulates in sympathetic nerves after monoamine oxidase inhibition and is released following neural stimulation (Kopin *et al.*, 1964, 1965; Fischer, Horst & Kopin, 1965; Baldessarini, 1971; Baldessarini & Vogt, 1972). Additionally, octopamine has a subcellular distribution similar to that of noradrenaline (Musacchio, Kopin & Snyder, 1964; Snyder, Michaelson & Musacchio, 1964; Snyder, Glowinski & Axelrod, 1965; Baldessarini & Vogt, 1971; Molinoff & Axelrod, 1972), and a physiological role for octopamine as a cotransmitter with noradrenaline has been proposed (Molinoff & Axelrod, 1969, 1972; Axelrod & Saavedra, 1977).

Fig. 1. Primary and alternative pathways for the formation of catecholamines: (1) tyrosine hydroxylase; (2) aromatic aminoacid decarboxylase; (3) dopamine- β -oxidase; (4) phenylethanolamine-*N*-methyl transferase; (5) nonspecific *N*-methyltransferase; (6) catechol-forming enzyme. Solid lines denote primary pathway. (Modified from Axelrod, 1963; and Cooper, Bloom & Roth, 1982).

The physiological significance of the alternative pathway catecholamines with respect to luteal function is unknown. It was the purpose of this study to examine the ability of the alternative pathway catecholamines tyramine, octopamine, synephrine and deoxyadrenaline to regulate steroidogenesis by bovine luteal cells.

Materials and Methods

Materials, drug description and preparation

Tyramine HCl, DL-octopamine HCl, DL-synephrine, deoxyadrenaline HCl, L-noradrenaline HCl, DL-adrenaline and DL-propranolol HCl were purchased from Sigma Chemical Co., St Louis, MO. Phentolamine HCl was donated by Ciba-Geigy Corp., Summit, NJ. Ham's F12 culture medium was purchased from Grand Island Biological Co., Grand Island, NY. The collagenase was Worthington, Type 1, Freehold, NJ, and *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (Hepes) was purchased from Calbiochem-Behring, San Diego, CA.

Propranolol is a nonspecific β -adrenergic receptor antagonist and phentolamine is a nonspecific α -adrenergic antagonist. All hormones and drugs were prepared in cold 0.05 M-phosphate-buffered saline (pH 7.2) before use in each experiment. In all cases *N* equals the number of animals tested. Duplicate determination of all treatments was performed for each corpus luteum.

Experimental procedures

Corpora lutea (CL) were obtained *per vaginam* from regularly cycling, nonlactating dairy cows on Days 10–12 of the oestrous cycle (oestrus = Day 0). Luteal tissue was placed into Ham's F12 culture medium containing 24 mM-Hepes (pH 7.4) at 4°C for transport to the laboratory. Luteal tissue was dissociated with collagenase (2000 u/g tissue) and cell number and viability were determined as described by Pate & Condon (1982). Incubations were conducted utilizing 2.5×10^5 viable cells in a final volume of 1 ml Ham's F12 culture medium and incubated for 2 h at 37°C as previously described (Battista & Condon, 1986). Tyramine, octopamine, synephrine and deoxyadrenaline were added to luteal cell suspensions at a final concentration of 10^{-6} to 10^{-3} M. Noradrenaline and adrenaline were tested at 10^{-7} to 10^{-4} M. For determination of the adrenergic receptor mediating the catecholamine response, noradrenaline and adrenaline (10^{-6} M) or

octopamine, synephrine and deoxyadrenaline (10^{-4} M) were added to incubations which had been preincubated for 30 min with propranolol (10^{-5} M) or phentolamine (10^{-5} M).

Progesterone in the incubation medium was quantitated by radioimmunoassay of unextracted samples using antiprogestrone-11-bovine serum albumin (No. 337, Niswender). The progesterone tracer used was [1,2- 3 H]progesterone (New England Nuclear, Boston, MA). The sensitivity of the assay, as determined by the lower 95% confidence limit of the maximum binding in the absence of any unlabelled progesterone, was 0.1 ng. The intra-assay and interassay coefficients of variability were 7.3% and 12.4% respectively. All standards were assayed in quadruplicate, and all unknowns were assayed in duplicate.

Statistical analysis

Analysis of difference between treatment means was conducted using one-way analysis of variance followed by the Student–Newman–Keuls mean separation procedure.

Results

Luteal steroidogenesis in the presence of alternative pathway catecholamines is shown in Fig. 2(a). Octopamine, synephrine and deoxyadrenaline significantly enhanced progesterone production at

Fig. 2. Effect of (a) alternative pathway catecholamines and (b) noradrenaline and adrenaline on progesterone production by dissociated luteal cells. Data are expressed as the mean $\pm$ s.e.m. ($N = 6$ for all treatments). Solid line denotes control value of 46.4 ± 2.13 ng/ml. Bars within treatments with different superscript letters are significantly different ($P < 0.05$).

concentrations of 10^{-6} to 10^{-3} M ($P < 0.05$). Tyramine was ineffective in regulating the production of progesterone ($P > 0.05$). The response to octopamine, synephrine and deoxyadrenaline was similar within each treatment concentration. Addition of noradrenaline and adrenaline at 10^{-7} to 10^{-4} M resulted in a significant elevation in the production of progesterone ($P < 0.05$, Fig. 2(b)). Comparison of the response of luteal cells to primary and alternative pathway catecholamines showed that noradrenaline and adrenaline were more potent agonists than were octopamine, synephrine or deoxyadrenaline ($P < 0.05$). The stimulatory effects of noradrenaline and adrenaline (10^{-6} M) and of octopamine, synephrine and deoxyadrenaline (10^{-4} M) were inhibited by propranolol (10^{-5} M, $P < 0.05$, Table 1) but not by phentolamine (10^{-5} M, $P > 0.05$). Neither propranolol nor phentolamine significantly altered basal progesterone production (data not shown).

Table 1. Effect of α - and β -adrenergic antagonists on primary and alternative pathway catecholamine-induced progesterone production (ng/ml incubation medium)

	N	Octopamine	Synephrine	Deoxyadrenaline	Noradrenaline	Adrenaline
Agonist alone	6	90.6 $\pm$ 2.25 ^a	87.4 $\pm$ 4.81 ^a	96.5 $\pm$ 1.92 ^a	99.9 $\pm$ 6.61 ^a	107.2 $\pm$ 4.07 ^a
Agonist + propranolol	4	57.8 $\pm$ 8.54 ^b	61.4 $\pm$ 5.77 ^b	52.3 $\pm$ 6.18 ^b	55.5 $\pm$ 3.30 ^b	63.8 $\pm$ 6.64 ^b
Agonist + phentolamine	4	93.9 $\pm$ 4.87 ^a	87.9 $\pm$ 7.88 ^a	89.8 $\pm$ 5.42 ^a	89.3 $\pm$ 6.18 ^a	98.7 $\pm$ 4.34 ^a

Values are mean $\pm$ s.e.m.

Means within columns with different superscript letters are significantly different ($P < 0.05$).

Control value equals 46.4 $\pm$ 2.13.

Discussion

These results demonstrate a regulatory role for the alternative pathway catecholamines octopamine, synephrine and deoxyadrenaline in the control of bovine luteal steroidogenesis. The stimulatory effects of noradrenaline and adrenaline on progesterone production and the ability of the β -adrenergic antagonist propranolol to inhibit this response is consistent with previous results (Condon & Black, 1976; Jordan *et al.*, 1978; Harwood *et al.*, 1980; Ratner *et al.*, 1980; Norjavaara *et al.*, 1982). In other mammalian tissues octopamine has been reported to interact with α - and β -adrenergic receptors (Fujiwara, Keisuke, Hideo, Takashi & Yoshiko, 1968), exclusively with α -receptors (Kelly & Burks, 1974; Kleinrok, 1979) or exclusively with β -adrenergic receptors (Chiba, 1976). The ability of propranolol but not phentolamine to inhibit the steroidogenic response to octopamine, synephrine and deoxyadrenaline suggests that their action is mediated by β -adrenergic receptors. The lower potency observed for the alternative pathway catecholamines with respect to progesterone production is similar to that reported for their ability to regulate other physiological processes (Lands & Grant, 1952; Fujiwara *et al.*, 1968; Nedergaard & Westermann, 1968; Kelly & Burks, 1974).

The mammalian ovary is extensively innervated with adrenergic and cholinergic nerve fibres, although the CL appears to be devoid of any direct neural innervation (Bahr, Kao & Nalbandov, 1974; Burden, 1978; Stefenson, Owman, Sjoberg, Sporrang & Walles, 1981). No data are presently available to suggest that the bovine CL can synthesize or retain catecholamines. HPLC analysis of bovine luteal tissue did not reveal noradrenaline or adrenaline to be present in early, mid or late cycle CL (P. J. Battista, C. Rexroad & W. A. Condon, unpublished observations). The failure to identify these catecholamines in bovine luteal tissue may suggest that the steroidogenic action of octopamine, synephrine and deoxyadrenaline is not due to conversion to primary catecholamines.

Additionally, the inability of tyramine to stimulate luteal steroidogenesis may suggest that the enzymes responsible for primary and alternative pathway catecholamine synthesis are not present in bovine luteal tissue.

The mechanism whereby catecholamines regulate progesterone production in the cow CL is unknown. In rat granulosa cells, adrenergic agents increase the enzymic activity of 3β -hydroxysteroid dehydrogenase while decreasing 20α -hydroxysteroid dehydrogenase activity (Hsueh *et al.*, 1983). Ovarian denervation or chemical sympathectomy decreases 3β -hydroxysteroid dehydrogenase activity in the rat CL, suggesting that catecholamines may regulate steroidal enzyme activity *in vivo* (Burden, 1978).

In summary, these results demonstrate that the alternative pathway catecholamines octopamine, synephrine and deoxyadrenaline stimulate steroid biosynthesis in bovine luteal tissue *in vitro*. The response to these catecholamines is less than that observed for noradrenaline and adrenaline, and the steroidogenic response of octopamine, synephrine and deoxyadrenaline is mediated by β -adrenergic receptors.

Scientific Contribution No. 1406 from the New Hampshire Agricultural Experiment Station. We thank Dr G. D. Niswender for the progesterone antiserum and D. Stranger for manuscript preparation. This research was a contribution to Northeast Regional Project NE-72.

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Received 17 February 1986