British Journal of Pharmacology (1996) 118, 1669-1674

Influence of dopamine as noradrenaline precursor on the secretory function of the bovine corpus luteum *in vitro*

[1]J. Kotwica, D. Skarzynski, M. Bogacki & G. Miszkiel

Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Division of Reproductive Endocrinology and Pathophysiology, 10-718 Olsztyn-Kortowo, Poland

1 Dopamine is assumed to affect the ovary function after its conversion into noradrenaline (NA).

2 To study this bovine luteal slices from 11-14 days of the oestrous cycle were preincubated for 24 h to recover β-receptors and next they were incubated for 1, 2 or 4 h with (a) different doses of dopamine; (b) dopamine together with a β-antagonist (propranolol) or with a dopamine receptor blocker (droperidol); (c) dopamine with a dopamine-β-hydroxylase inhibitor.

3 Dopamine stimulated the luteal content of oxytocin (OT) and progesterone. This effect was inhibited by propanolol but not by droperidol.

4 Dopamine added to the medium was followed by an increase of noradrenaline there. This rise was dose and time-dependent.

5 The dopamine-β-hydroxylase inhibitor, inhibited the stimulating effect of dopamine on luteal progesterone and OT content.

6 Bovine corpus luteum can synthesize *de novo* NA from dopamine as a precursor.

Keywords: Corpus luteum; noradrenaline; dopamine; progesterone; β-receptors; oxytocin

Introduction

Dopamine stimulates oxytocin (OT) release from the pituitary of lactating rats, with a potency comparable to that of noradrenaline (NA) (Clarke *et al.*, 1979). Results from studies on other species have suggested that dopamine can affect this process directly through specific dopamine receptors (Clarke *et al.*, 1979; Hilditch & Drew, 1985; Cameron *et al.*, 1992), indirectly through the adrenoceptors by way of a cross-reaction (Weiner, 1985) or finally dopamine can be converted to NA in the process catalysed by dopamine-β-hydroxylase (Levin *et al.*, 1960). Dopamine, which is a precursor for NA, was discovered to be present in bovine corpus luteum (CL) (Battista *et al.*, 1989; Denning-Kendall *et al.*, 1991) and was found to stimulate OT secretion from CL in conscious heifers (Kotwica *et al.*, 1995). NA influences the concomitant secretion of progesterone and ovarian OT during all stages of oestrous cycle in cattle (Kotwica *et al.*, 1991; Kotwica & Skarzynski, 1993; Jaroszewski & Kotwica, 1994) by acting through the β-adrenoceptors (Godkin *et al.*, 1977; Skarzynski & Kotwica, 1993). Therefore dopamine is assumed to affect the ovarian function after its conversion into NA. In order to study this, a series of experiments using an *in vitro* model were performed.

Methods

Corpora lutea collection and slices preparation

Corpora lutea (CL) from 11-14 days of the oestrous cycle were collected from the commercial slaughterhouse within 15-20 min of death, immediately placed on the ice-cold medium (M-199) containing 10% calf serum, penicillin (10 u ml^{-1}), streptomycin (100 μg ml^{-1}), amphotericin (2 μg ml^{-1}) and L-glutamine (100 μg ml^{-1}). Luteal tissue was chopped with scissors (slices 0.1-1 mm^3) in sterile conditions and washed 3 times before being subjected to incubation.

[1]Author for correspondence.

Preliminary experiment

Our earlier studies have shown that pre-slaughter stress causes almost complete down-regulation of β-receptors in corpus luteum obtained from a commercial slaughterhouse (Pesta *et al.*, 1994). Since, in present studies we used ovaries from the slaughterhouse we wanted to determine within what time after slaughtering the β-receptors in the CL recover. To obtain luteal cells, corpora lutea were mechanically dispersed by cell dissociation equipment (Sigma Co.). Next cells (500 000 cells/vial) were pre-incubated for 5, 10, 15, 24, 36 and 48 h ($n=6$ of each) and then incubated for 45 min (Pesta *et al.*, 1994) with either 10^5 d.p.m. (125 pg) [^{3}H]-dihydroalprenolol (Amersham) or [^{3}H]-dihydroalprenolol plus 1000 fold excess (125 ng) of alprenolol (Sigma). Specific binding was calculated.

Experiment 1

This experiment was performed to establish the effective dose of dopamine. On the basis of data from preliminary studies, slices (100 mg) from bovine CL ($n=12$) suspended in 3 ml of M-199 were pre-incubated for 24 h at 38°C in a humidified 5% CO_2 atmosphere each in 4 replicates. Then the medium was replaced with fresh M-199 supplemented with luteinizing hormone (LH; 100 ng ml^{-1} positive control) or with five different amounts of dopamine (10^{-3}-10^{-7} M). After 1 h, 2 h and 4 h of incubation the luteal slices were collected for progesterone and OT determination. Furthermore, the dopamine and NA content were determined in luteal slices treated with 10^{-3}, 10^{-4}, and 10^{-5} M dopamine.

Experiment 2

For determining the receptor type mediating the CL responses to dopamine, luteal slices were pre-incubated for 1 h with 10^{-4} M propranolol (β-antagonist) or with 10^{-4} M droperidol (dopamine receptor blocker) for 30 min. The dopamine was added at a final concentration of 10^{-5} M. Slices were collected after 60 min of incubation.

Experiment 3

To study whether CL can itself synthesize NA from dopamine as a precursor, luteal slices were treated with dopamine (10^{-5} M) either alone or after pretreatment for 30 min with a dopamine-β-hydroxylase inhibitor (3-phenylpropargylamine hydrochloride; 10^{-5} M) (Research Biochemical International, U.S.A.). Medium and luteal slices were collected for hormone determinations.

Homogenization of luteal slices

After incubation luteal slices were deeply frozen in liquid nitrogen and homogenized (powdered) in a Vibratory Mill (Retsch MM-2). Next, from powdered tissue, OT and progesterone were extracted as described previously by Tsang *et al.* (1990). Recovery for OT and progesterone averaged 90% and 84%, respectively. Data were corrected for procedural loses.

Hormone determinations

Progesterone concentrations were determined by radioimmunoassay (RA; Kotwica *et al.*, 1990). Rabbit progesterone antiserum (IFP4) had been characterized previously (Kotwica *et al.*, 1994). The sensitivity of the procedure was 15 pg per tube. Intra- and interassay variations were 7.4 and 13.9% respectively. The relationships between real (x) and determined (y) amount of four different concentrations of progesterone added to the plasma samples is expressed by the linear regression equation ($y = 1.034 \times -0.13$).

OT plasma level was determined by using ice-chilled acetone 3 times the volume of the extraction. The hormone was iodinated with 5 μCi of ^{125}I by means of chloramine (Greenwood *et al.*, 1963); OT (10 μg) and chloramine (10 μg) were incubated for 40 s at room temperature with gentle mixing. The reaction was interrupted by adding 50 μg sodium metabisulphite. Iodination products were separated on Sephadex (G-50) column (1 × 35 cm). Rabbit OT antiserum (R-1) had been characterized previously (Kotwica & Skarzynski, 1993). The efficiency of extraction was 85% and final data were corrected for procedural losses. The sensitivity of the method was 3 pg ml^{-1}. Intra- and interassay variations were 7.5 and 14.6%, respectively. The precision of the procedure is expressed by the linear regression equation ($y = 0.99x + 0.14$).

Tissue concentrations of NA and dopamine were determined by high performance liquid chromatography (h.p.l.c.) with electrochemical detection (HP 1049A; Hewlett-Packard). Catecholamines were extracted from the powdered luteal slices with a 10 fold excess amount of 0.1 M trichloracetic acid (TCA) containing 0.01% $Na_2S_2O_5$ and 0.0003% ascorbic acid. This suspension was mixed by means of an Ultra-Turrax (T25, IKA). Next the suspension was centrifuged for 20 min at 14000 × *g* and 500 μl of the supernatant was taken and diluted with 1 ml of Tris buffer, pH 8.6. The further procedure was as described previously (Kotwica *et al.*, 1996). The only changes were 10 ng of isoprenaline was used as an external standard, the time of catecholamine absorption was prolonged to 17 min, cathecholamine standards were prepared in 0.5 ml of 0.1 M TCA containing 0.01% $Na_2S_2O_5$ and 0.0003% ascorbic acid and, finally, the mobile phase had a pH = 3.5. Recovery from this procedure was 71% and the final data were corrected for procedural losses.

Statistical analysis

The secretion of OT and progesterone after dopamine treatment was measured by comparing values after dopamine treatment with control values. The influence of time of incubation and the dose of dopamine on tissue content of noradrenaline was described by linear regression. The differences

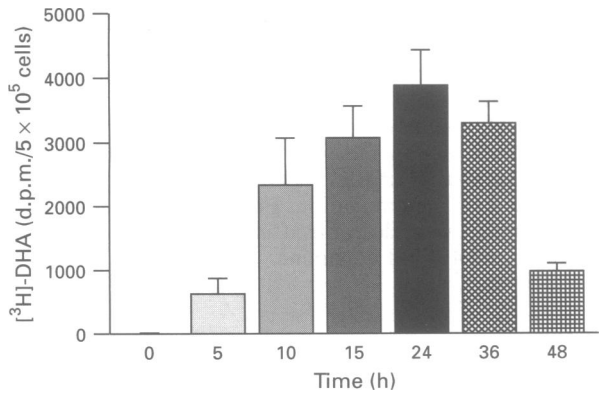

Figure 1 Specific binding of [^{3}H]-dihydroalprenolol (DHA) by bovine dispersed luteal cells. [^{3}H]-dihydroalprenolol, 10^5 d.p.m. (125 pg) was incubated together with 1000 fold excess (125 ng) of cold alprenolol. Columns represent means and vertical lines show s.e.mean ($n = 6$).

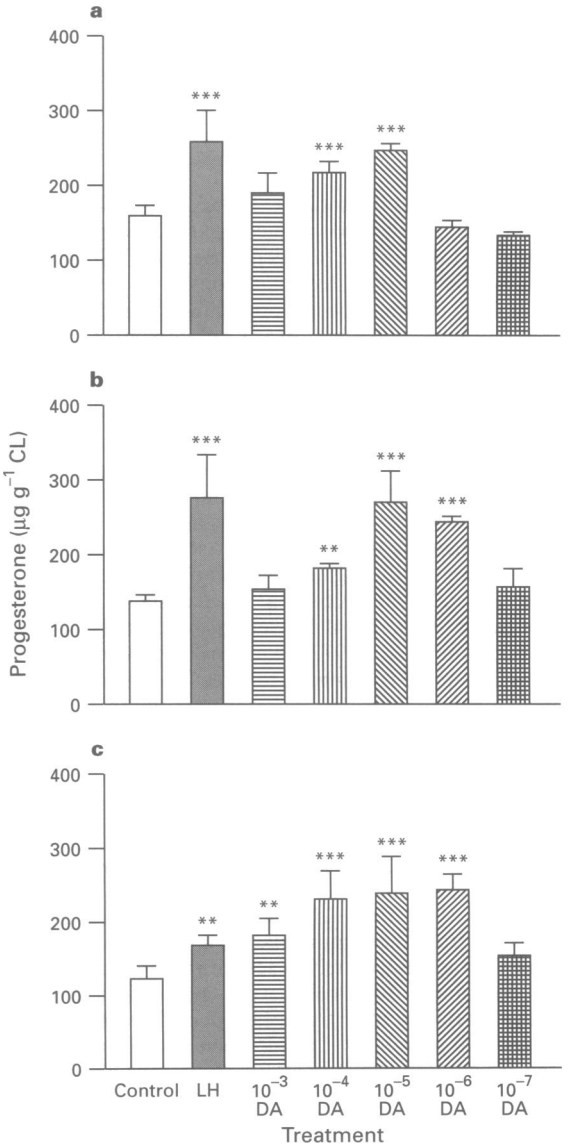

Figure 2 Progesterone content of luteal slices ($n = 16$) after (a) 1 h, (b) 2 h and (c) 4 h of incubation with luteinizing hormone (LH; 100 mg ml^{-1}) and different doses of dopamine (DA). $^{**}P < 0.01$; $^{***}P < 0.001$; significantly different from control.

between mean (±s.e.mean) values were determined by one-way analysis of variance and $P<0.05$ was accepted as significant (InStat, GraphPAD).

Results

Preliminary experiments

From 0 to 24 h of incubation [^{3}H]-dihydroalprenolol specific binding to luteal slices increased in a time-dependent manner (Figure 1). The highest specific binding was after 24 h of incubation and it amounted to about 4% of total titrated dihydroalprenolol. Then the specific binding gradually decreased. Hence in all further experiments luteal slices were pre-incubated for 24 h without any supplements.

Experiment 1

LH increased progesterone concentrations in luteal slices after 1 h ($P<0.05$), 2 h and 4 h ($P<0.01$) of incubation indicating that the luteal slices were reactive during the whole experimental period. The increase in progesterone concentration in luteal slices was most evident after treatment with 10^{-4} M and 10^{-5} M of dopamine after 1 and 2 h of incubation. All but the lowest dose of dopamine used increased progesterone concentration in luteal slices after 4 h of incubation (Figure 2); 10^{-5} M dopamine increased OT content ($P<0.01$) in luteal slices after only 1 h of stimulation (Figure 3). Therefore, for further studies 10^{-5} M dopamine was selected even though the concentrations of dopamine used increased ($P<0.01$) NA concentration in luteal tissue (Figure 4). The decreased dopamine concentration in luteal tissue during incubation was followed by an increase of NA after medium supplementation with 10^{-3} and 10^{-4} M dopamine ($r=-0.56$; $P<0.056$) (Figure 4). There was also a correlation between NA tissue concentration and dose of dopamine in the medium (Figure 5).

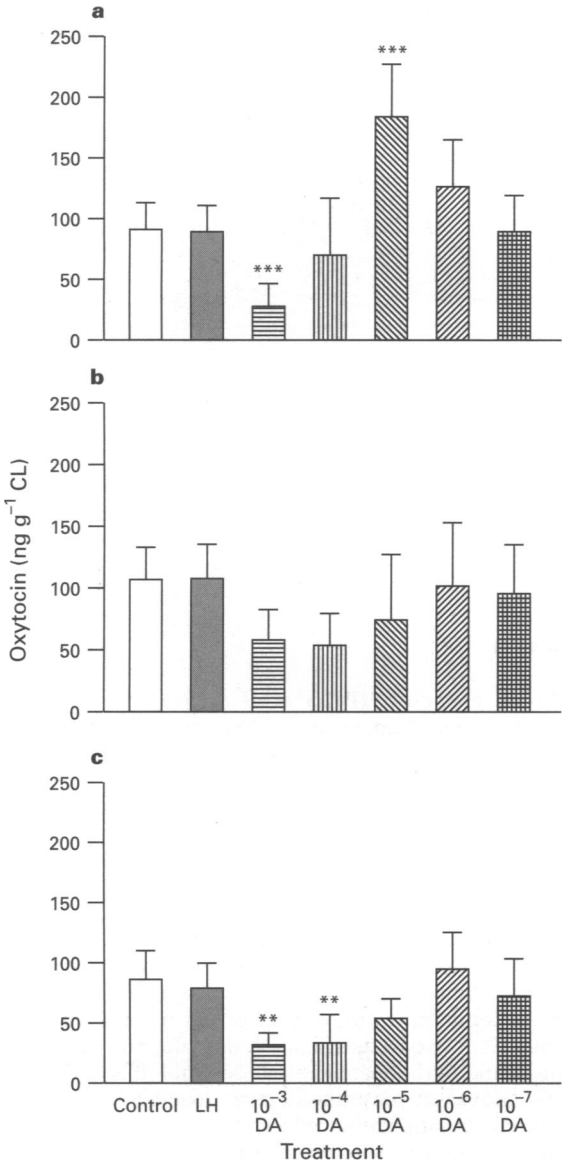

Figure 3 Mean content of oxytocin in luteal slices ($n=16$) after (a) 1 h, (b) 2 h and (c) 4 h of incubation with luteinizing hormone (LH; 100 mg ml^{-1}) and different doses of dopamine (DA). Vertical lines show s.e.mean. **$P<0.01$; ***$P<0.001$, significantly different from control.

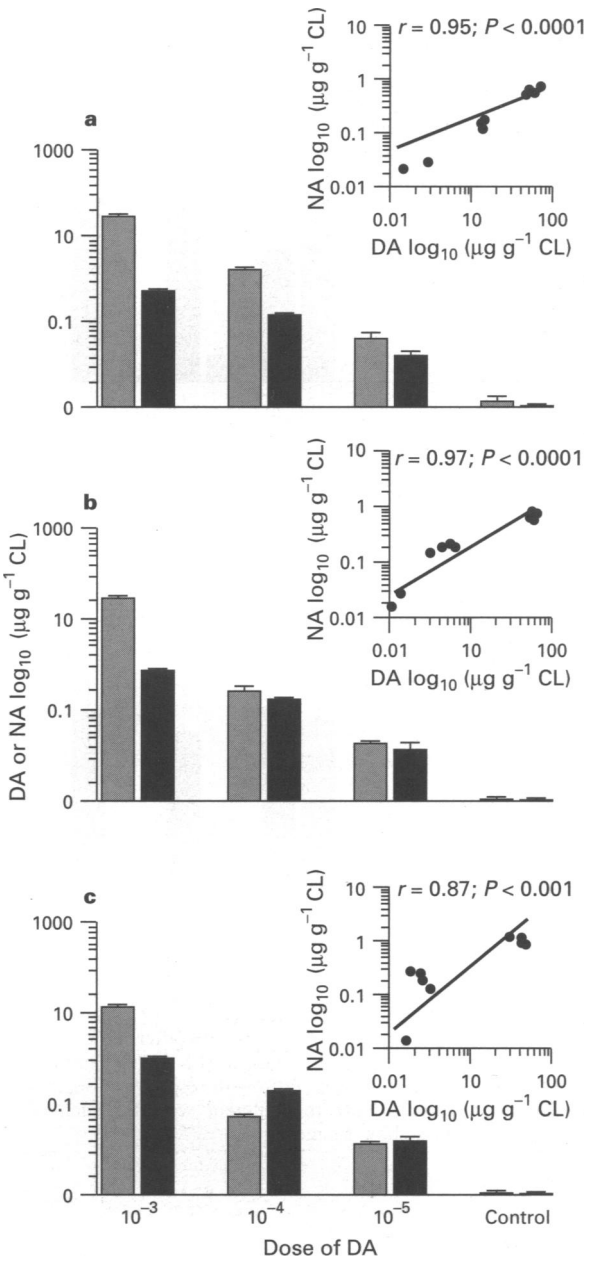

Figure 4 Relationship between dopamine (DA) and NA content in luteal slices ($n=16$) depending upon dose of dopamine added into the incubation medium after (a) 1 h, (b) 2 h and (c) 4 h of incubation. Effect of dopamine dose on NA content in luteal tissue is illustrated by linear regression (insets). Stippled columns, dopamine content; solid columns, NA content.

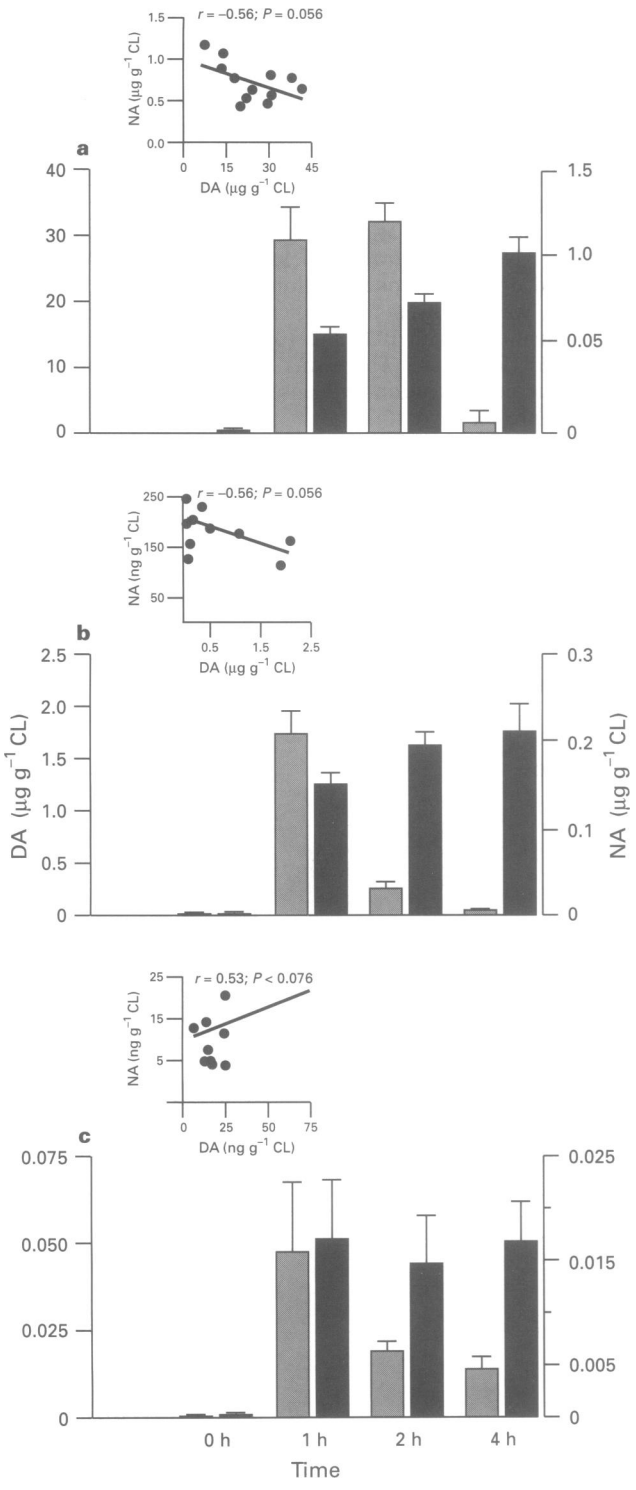

Figure 5 Mean content of dopamine (DA, stippled columns) and NA (solid columns) in luteal slices ($n=16$) after 1, 2 and 4 h of incubation with (a) 10^{-3}, (b) 10^{-4} and (c) 10^{-5} M dopamine. Linear regression for the NA: dopamine ratio with respect to dopamine dose is shown in the insets. Note the different scale for each of three graphs. Vertical lines show s.e.mean.

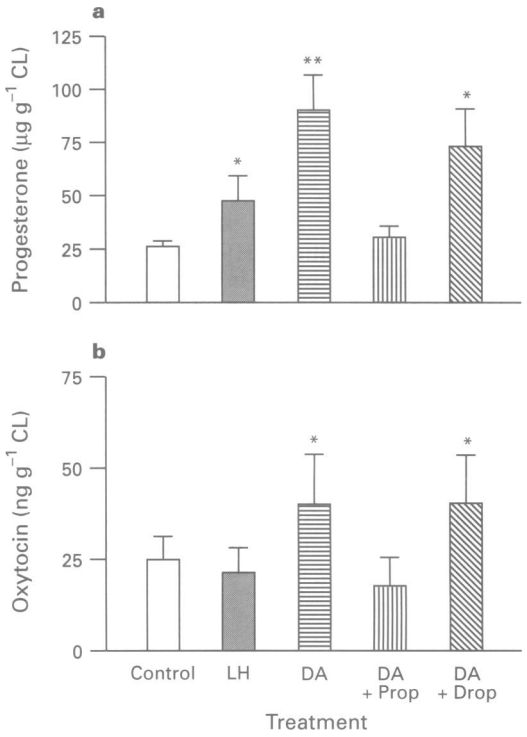

Figure 6 Mean luteal content ($n=24$) of progesterone (a) and oxytocin (b) after 1 h incubation with luteinizing hormone (LH, 100 ng ml^{-1}), dopamine (DA; 10^{-5} M) together with propranolol (Prop, 10^{-4} M) or with droperidol (Drop; 10^{-4} M). Vertical lines show s.e.mean. *$P<0.05$; **$P<0.01$ different from control value respectively.

Experiment 2

The increased luteal content of both progesterone ($P<0.01$) and oxytocin ($P<0.05$) induced by dopamine was inhibited by medium supplementation with propranolol but not droperidol (Figure 6).

Experiment 3

The dopamine-β-hydroxylase inhibitor (3-phenylpropargylamine hydrochloride) added to the incubation medium significantly inhibited the increased concentrations of progesterone ($P<0.01$) and oxytocin ($P<0.05$) induced by dopamine in the CL (Figure 7a). Hormonal changes in the medium were less evident (Figure 7b). This was the main reason why tissue hormone concentrations were measured.

Discussion

We found previously (Pesta *et al.*, 1994) that pre-slaughter stress (transportation, new surroundings, fasting etc.) in commercial slaughterhouses down-regulates luteal β-receptors. The present preliminary studies revealed that maximal recovery of β-receptors in luteal cells was observed after 24 h. Studies *in vivo* have shown that NA affects ovarian function through an effect on β-receptors (Godkin *et al.*, 1977; Skarzynski & Kotwica, 1993). Hence ovaries obtained from the slaughterhouse can be a useful model to study the effect of catecholamines on CL function. Dopamine stimulated the content of progesterone and OT in luteal slices within 1 h of incubation. Since the dopamine receptor blocker (droperidol) given concomitantly with dopamine did not prevent this stimulant effect, we conclude that CL does not possess dopamine receptors and that dopamine itself does not affect CL function directly. However, propranolol given together with dopamine diminished its stimulating effect (Figure 6). Therefore, it is assumed dopamine has to be converted to NA to influence CL function, as shown by use of a dopamine-β-hydroxylase blocker (Figure 7). We cannot state precisely after what time of incubation dopamine affects CL. NA (0.3 μg min^{-1} kg^{-1}) infused into the aorta abdominalis in conscious heifers has been shown to increase ovarian OT concentrations in blood after

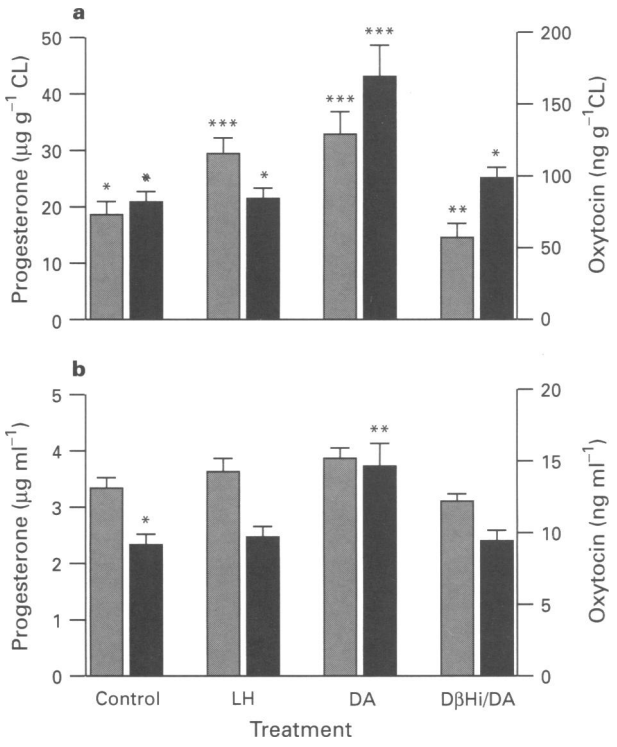

Figure 7 Mean content of progesterone (stippled columns) and oxytocin (solid columns) in the luteal tissue (a) and in medium (b). Medium was supplemented with luteinizing hormone (LH; 100 ng ml^{-1}), dopamine (DA; 10^{-5} M) or dopamine together with the dopamine-β-hydroxylase inhibitor (3-phenylpropargylamine hydrochloride; DBHi) (10^{-5} M). Vertical lines show s.e.mean. *$P<0.05$, **$P<0.01$, ***$P<0.001$ different from control value, respectively.

2–5 min (Kotwica *et al.*, 1991). However, in the present study hormones were measured in luteal tissue no earlier than 1 h after incubation.

Although dopamine was found to be present in bovine CL its origin is not clear. Battista *et al.* (1989) and Denning-Kendall *et al.* (1991) assumed that mast cells, stromal adrenergic nerves or the peripheral circulation is a source of dopamine which can be preferentially taken up by luteal cells. However, these authors proposed that dopamine cannot be synthesized within the CL. Rich adrenergic innervation of all parts of the ovary, i.e. adventitia, blood vessels, ovarian medulla, follicles, CL capsules (Burden, 1978; Stefenson *et al.*, 1981; Sorger *et al.*, 1983) supports this view. In nerve fibres, dopamine-β-hydroxylase produces NA from its precursor dopamine. With luteal slices in the present study we utilized, supposedly, dopamine-β-hydroxylase from neural fibres, but this model does not allow us to exclude the presence of this enzyme in other parts of the CL including luteal tissue.

Nevertheless, these studies point out not only the ability of CL to synthesize *de novo* NA but also confirm its importance in CL function. We showed that constant β-adrenoceptor-mediated stimulation of the CL ensures basal secretion of progesterone, since short-term blockade of β-adrenoceptors by propranolol immediately decreased peripheral progesterone concentrations by 20–40% (Kotwica *et al.*, 1991). On the other hand, short-term activation of the noradrenergic system significantly stimulated OT and progesterone secretion within a few minutes, suggesting an efficient luteotrophic influence of the noradrenergic system, important especially in stressful situations occurring during the early stages of pregnancy (Kotwica & Skarzynski, 1993; Kotwica *et al.*, 1995). Therefore the ability of CL to synthesize NA quickly and in large amounts indicates this mechanism of CL protection operates with high efficiency.

Battista *et al.* (1989) found concentrations of 40 ng and 10 ng of dopamine and NA respectively, per gram of bovine CL. Data obtained by Denning-Kendall *et al.* (1991) were similar. In the present study the amount of NA measured in luteal tissue was dependent ($r=0.87-0.95$; $P<0.001$) on the dose of dopamine added to the medium (Figure 4) and also upon the time of treatment (Figure 5). These results suggest that the CL has the ability to synthesize NA to concentrations much higher than those obtained physiologically in this tissue. The decrease in the dopamine content in luteal slices during incubation was negatively correlated ($r=-0.56$; $P<0.05$) with NA luteal content if the medium was supplemented with 10^{-3} M and 10^{-4} M dopamine. The lack of a similar relationship with 10^{-5} M dopamine is probably due to the lower NA synthesis on the one hand, and its consumption by luteal tissue on the other hand. Moreover, CL catecholamines can be catabolized by monoamine oxidase, as shown in rats (Yoshimoto *et al.*, 1986). These processes could mask the increased NA synthesis after medium supplementation with the lower (10^{-5} M) dose of dopamine (Figures 4 and 5).

In conclusion, dopamine is, in the CL, a direct substrate for synthesis of NA which can then stimulate secretion of ovarian OT and progesterone. CL contains dopamine-β-hydroxylase an enzyme which produces NA from its precursor dopamine present also in CL. Thus bovine CL contains both dopamine and dopamine-β-hydroxylase and is a place of NA synthesis both of which support the secretory function of the CL.

This study was supported by grants from US-Polish Joint Found II (USDA/MR-92-94) and from Polish Academy of Sciences. Progesterone (IFZ-4) and oxytocin (R-4) antiserum were donated by S. Okrasa and G. Kotwica respectively (University of Agriculture and Technology, Olsztyn, Poland). Ovine LH (APF9360B) was a gift from Dr A.P. Parlow (Pituitary Hormones & Antisera Center, Torrance, CA).

References

BATTISTA, P.J., REXROAD, C.E. Jr, POFF, J.P. & CONDON, W.A. (1989). Support for a physiological role of endogenous catecholamines in the stimulation of bovine luteal progesterone production. *Biol. Reprod.*, **41**, 801–812.

BURDEN, H.W. (1978). Ovarian innervation. In *The Vertebrate Ovary*. ed. Jones, R.E. pp. 615–638. New York: Plenum Press.

CAMERON, J.L., POMERANTZ, S.M., LAYDEN, L.M. & AMICO, J.A. (1992). Dopaminergic stimulation of oxytocin concentrations in the plasma of male and female monkeys by apomorphine and D_2 receptor agonist. *J. Clin. Endocrinol. Metab.*, **75**, 855–860.

CLARKE, G., LINCOLN, D.W. & MERRICK, L.P. (1979). Dopaminergic control of oxytocin release in lactating rats. *J. Endocrinol.*, **83**, 409–420.

DENNING-KENDALL, P.A., WILD, M.L. & WATHES, D.C. (1991). Regional differences in catecholamine concentrations in bovine ovaries analysed by high-performance liquid chromatography. *J. Endocrinol.*, **129**, 221–226.

GODKIN, J.D., BLACK, D.L. & DUBY, R.T. (1977). Stimulation of cyclic AMP and progesterone synthesis by LH, PGE_2 and isoproterenol in the bovine CL in vitro. *Biol. Reprod.*, **17**, 514–518.

GREENWOOD, F.C., HUNTER, W.M. & GLOVER, J.S. (1963). The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. *Biochem. J.*, **89**, 114–121.

HILDITIH, A. & DREW, G.M. (1985). Peripheral dopamine receptor subtypes - a close look. *Trends Pharmacol. Sci.*, **6,** 396–400.

JAROSZEWSKI, J. & KOTWICA, J. (1994). Reduction of oxytocin content in corpus luteum does not affect the duration of estrous cycle in cattle. *Reprod. Nutr. Develop.*, **34,** 175–182.

KOTWICA, J., SKARZYNSKI, D. & JAROSZEWSKI, J. (1990). Coccygeal artery as a route for the administration of drugs into the reproductive tract of cattle. *Vet. Rec.*, **127,** 38–40.

KOTWICA, J., SKARZYNSKI, D., JAROSZEWSKI, J. & KOTWICA, G. (1991a). Effect of norepinephrine on the release of progesterone and ovarian oxytocin in cattle. *Anim. Reprod. Sci.*, **26,** 179–191.

KOTWICA, J., SKARZYNSKI, D. & JAROSZEWSKI, J. (1991b). Involvement of β-adrenoceptors in the regulation of luteal function in cattle. *Br. Vet. J.*, **147,** 189–196.

KOTWICA, J. & SKARZYNSKI, D. (1993). Influence of oxytocin removal from corpus luteum on secretory function and duration of the oestrous cycle in cattle. *J. Reprod. Fert.*, **97,** 411–417.

KOTWICA, J., SKARZYNSKI, D., JAROSZEWSKI, J. & BOGACKI, M. (1994). Noradrenaline affects secretory functions of corpus luteum independently of prostaglandins in conscious cattle. *Prostaglandins*, **48,** 1–10.

KOTWICA, J., SKARZYNSKI, D. & BOGACKI, M. (1995). Effect of catecholamines on the secretory function of bovine corpus luteum. *Dom. Anim. Reprod.*, **30,** 218–223.

KOTWICA, J., SKARZYNSKI, D., BOGACKI, M. & JAROSZEWSKI, J. (1996). Role of dopamine in the secretory function of corpus luteum in cattle. *J. Physiol. Pharmacol.*, **47,** (in press).

LEVIN, E.Y., LEVENBERG, B. & KAUFMAN, S. (1960). The enzymatic conversion of 3,4-dihydroxyphenylethylamine to norepinephrine. *J. Biol. Chem.*, **235,** 2080–2086.

PESTA, M., MUSZYNSKA, A., KUCHARSKI, J., SUPERATA, J. & KOTWICA, J. (1994). Beta adrenergic receptors in corpora lutea from different stages of the estrous cycle in conscious and slaughtered cattle. *Biol. Reprod.*, **50,** 215–221.

SKARZYNSKI, D. & KOTWICA, J. (1993). Mechanisms of noradrenaline influence on the secretion of ovarian oxytocin and progesterone in conscious cattle. *J. Reprod. Fert.*, **97,** 419–424.

STEFENSON, A., OWMAN, CH., SJOBERG, N-O., SPORRONG, B. & WALLES, B. (1981). Comparative study of the autonomic innervation of the mammalian ovary, with particular regard to the follicular system. *Cell Tissue Res.*, **215,** 47–62.

SORGER, T., PITTMAN, R. & SODERWALL, A.L. (1983). Principal features of the nerve supply to the ovary, oviduct and tubal third of the uterus in the golden hamster. *Biol. Reprod.*, **28,** 461–482.

TSANG, P.C.W., WALTON, J.S. & HANSEL, W. (199). Oxytocin-specific RNA, oxytocin and progesterone concentrations in corpora lutea of heifers treated with oxytocin. *J. Reprod. Fert.*, **89,** 77–84.

WEINER, N. (1985). Norepinephrine, epinephrine and the sympathomimetic amines. In *Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Goodman, L.S., Rall, T.W. & Murad, F. pp. 145–180. New York: Macmillan Publishing Co.

YOSHIMOTO, Y., SAKUMOTO, T., ARAI, R., MIYAKE, A., KIMURA, H., AONO, T., TANIZAWA, O. & MALADA, T. (1986). Monoamine oxidase in rat ovary during the estrous cycle. A histochemical study by a new coupled peroxidatic oxidation method. *Endocrinology*, **199,** 1800–1804.

(Received February 2, 1996
Revised March 25, 1996
Accepted April 11, 1996)