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Melatonin and serotonin regulate the release of insulin-like growth factor-I, oxytocin and progesterone by cultured human granulosa cells

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Summary: The direct influence of indoleamines on ovarian peptide hormones and growth factor secretion, in contrast to steroidogenesis, is yet to be throughly investigated. The aim of our in vitro experiments was to investigate the influence of melatonin and serotonin (5-hydroxytryptamine) (0.01–10 μg/ml) on the release of insulin-like growth factor-I (IGF-I), oxytocin and progesterone by cultured human granulosa cells. It was observed that both melatonin and serotonin stimulate IGF-I release. Melatonin also stimulated oxytocin output. Serotonin increased oxytocin secretion only at the highest dose (10 μg/ml). Both melatonin and serotonin were potent inhibitors of progesterone release. The present results suggest a possible involvement of the indoleamines melatonin and serotonin in the direct regulation of growth factor, nonapeptide and steroid hormone secretion by human ovarian cells.

Introduction

The regulation of reproductive processes by the indoleamines serotonin (5-HT) and melatonin through hypothalamic monoamines and luteinizing hormone releasing hormone (LH-RH) has been documented (Reiter,1980; Arendt,1986; Lecorre and Chemineau, 1993). Ovarian cycle-dependent variations in the levels of indoleamines occur in human blood (Kennaway et al., 1977; Blum et al., 1992; Hindberg and Naesh, 1992) and follicular fluid (Brzezinski et al., 1987; Bodis et al., 1993). Taken together with the presence of serotonin (5-HT) receptors in rat and human ovaries (Saundaram et al., 1993), this suggests a direct influence of indoleamines on the ovary.

There is some evidence for direct effects of indoleamines on steroid secretion by ovarian cells. Melatonin stimulated the release of progesterone, but not estradiol, from granulosa cells isolated from rat (Fiske et al., 1984), bovine (Webley and Luck, 1986), ovine (Baratta and Tamanini, 1992) and human (Webley and Luck, 1986; Brzezinski et al., 1991,1992) ovaries. In cultured porcine granulosa cells melatonin inhibited progesterone and stimulated estradiol output (Sirotkin, 1994). In contrast, progesterone secretion by ovine luteocytes did not change after melatonin treatment (Baratta and Tamanini, 1992). Serotonin was able to stimulate progesterone, testosterone and estradiol release by isolated hamster (Terranova et al., 1990) and rat (Tanaka et al., 1993) ovarian follicles, and progesterone production by cultured bovine luteal cells (Rhodes and Randall, 1982; Battista and Condon, 1986; Battista et al., 1987). The influence of serotonin on human ovarian cells has not been reported.

It is known that ovarian cells produce not only steroid, but also peptide hormones (in particular, oxytocin) and growth factors, which are potent regulators of ovarian steroidogenesis, corpus luteum development and oocyte maturation (Wathes, 1989; Giudice, 1992). Our previous experiments (Sirotkin, 1994) demonstrated an inhibitory influence of melatonin on oxytocin and vasopressin secretion by porcine granulosa cells. There are no reports of the involvement of indoleamines in the regulation of non-steroidal substances in human ovaries. In our experiments we have studied the influence of melatonin and serotonin on insulin-like growth factor-I (IGF-I), oxytocin and progesterone release by human granulosa cells in vitro.

Materials and methods

Preparation and culture of granulosa cells

Granulosa cells were aspirated from intact ovarian follicles, 1–7 mm in diameter without visible signs of atresia or pathology, 1–2 days after spontaneous ovulation, from women undergoing ovariectomy for cancer of the uterus. Granulosa cells were recovered from follicular fluid after centrifugation at 200 × g, and washed twice with sterile DMEM/F-10 1:1 mixture (Sigma, St.Louis, USA) supplemented with 10% heat-inactivated fetal bovine serum (Institute of Veterinary Medicine, Brno, Czech Republic) and 50 µg/ml gentamicin (Pharmachim, Sophia, Bulgaria). Cells were cultured at 37.5 °C in 5% CO_2 in humidified air in 24 chamber plates (Sarstedt, Vienna, Austria) at a concentration of 0.5–2 × 10^6 cells/ml medium/well. After two days of culture the medium was replaced using similar medium supplemented with 5-HT or melatonin (Sigma; 0, 0.01, 0.1, 1.0 or 10 µg/ml). Immediately before use, indoleamines were initially dissolved in absolute ethanol, and then diluted with the incubation medium such that the maximum final concentration of ethanol did not exceed 0.01% v/v. Control cells were cultured without exogenous indoleamines. After two days of culture the new medium was aspirated and stored at −20 °C to await assay. Cell number and viability (55–70%) were determined at this time by haemocytometer and Trypan blue exclusion and did not differ significantly between the groups.

Radioimmunoassay

In each sample of medium the levels of IGF-I, oxytocin and progesterone were determined in duplicate by RIA without extraction.

IGF-I concentrations were determined using kits from Mediagnost (Tübingen, Germany). This is the only radioimmunoassay which allows measurement of low amounts of IGF-I and of its fragments. To dissociate the IGF/IGF binding protein complex, the samples were diluted 1:5 in acidic buffer, and IGF binding protein was neutralized by excess recombinant hIGF-II. The cross-reactivity of antiserum to IGF-II was less than 0.05%. The assay standard was recombinant IGF-I devoid of methIGF-I and IGF-I variants with mismatched disulphide bonds. The sensitivity of the assay was 2 pg/ml. The inter- and intraassay coefficients of variation were 7.4 and 3.2% respectively.

Oxytocin levels were measured by kits from the Institute for Research, Production and Application of Radioisotopes (Prague, Czech Republic). Antiserum to oxytocin cross-reacted less than 0.005% with arginine-vasopressin, 0.04% with lysine-vasopressin, 17% with arginine-vasotocin and less than 0.01% with insulin, melatonin and serotonin. The maximal inter- and intraassay coefficients of variation were 12 and 10%, respectively.

Progesterone was determined using kits from the Institute of Radiooecology and Application of Nuclear Technics (Košice, Slovak Republic). The antiserum to progesterone cross-reacted 58.6% with 11-hydroxyprogesterone and less than 0.01% with testosterone, estradiol, insulin, melatonin and serotonin. The sensitivity was 2 pmol/ml. Inter- and intraassay coefficients of variation did not exceed 13 and 9%, respectively.

Statistics

The experiments were performed 3 times with different pools of granulosa cells harvested from 4–6 ovaries (2–3 patients) in replicates of 4 wells per treatment. In each experiment the amount of substance produced by the cells was calculated as a difference between the values determined in the medium cultured with and without (blank control) granulosa cells. The rates of secretion were expressed in the units given by the kit manufacturer per 10^6 viable cells/day, as means ± S.E.M. Experiments were compared using ANOVA, and thereafter subjected to Duncan's test to determine the significance of differences between control and treatment groups.

Results and discussion

Melatonin and 5-HT increased IGF-I concentration in the granulosa cell conditioned medium at doses of 0.1 and 1.0 and 10 µg/ml respectively (Fig. 1). Melatonin at doses of 0.1, 1.0 and 10 µg/ml also increased oxytocin release. Most doses of 5-HT did not influence oxytocin output, but the highest, 10 µg/ml, was inhibitory (Fig. 2). Melatonin at 0.01, 0.1 and 1.0 µg/ml, and serotonin at 0.01 and 0.1 µg/ml, reduced progesterone release, whilst higher doses of indoleamines were ineffective (Fig. 3).

Since low doses of both 5-HT and melatonin used in our experiments are comparable with the indoleamine concentrations in plasma (Kennaway et al., 1977; Reiter, 1980; Arendt, 1986; Blum et al., 1992; Hindberg and Naesh, 1992) and in follicular fluid (Brzezinski et al., 1987; Bodis et al., 1993), our observations in vitro may reflect processes that occur under the influence of indoleamines in vivo. Higher doses (1 or 10 µg/ml) may be considered to be supraphysiological and this may explain, why increaseing the doses of serotonin (Figs. 1, 3) and melatonin (Fig. 3) above 100 ng/ml caused cellular responses to fall or even disappear.

Our observations of inhibition of progesterone release after melatonin and serotonin treatments at low and middle doses differ from those of other authors (Fiske et al., 1984; Webley and Luck, 1986; Terranova et al., 1990; Brzezinski et al., 1991,1992; Baratta and

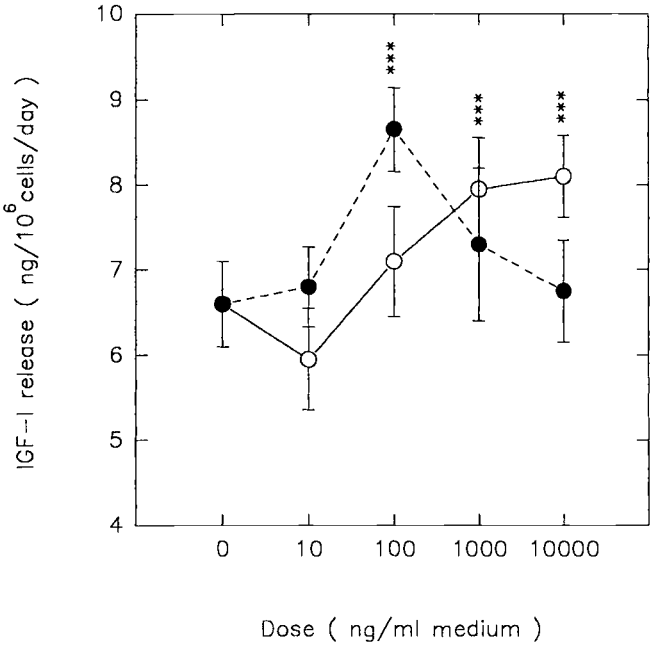

Fig. 1 Effects of melatonin (———) and serotonin (– – –) on IGF-I release by cultured human granulosa cells. Values are means ± S.E.M., *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ compared with control (without addition)

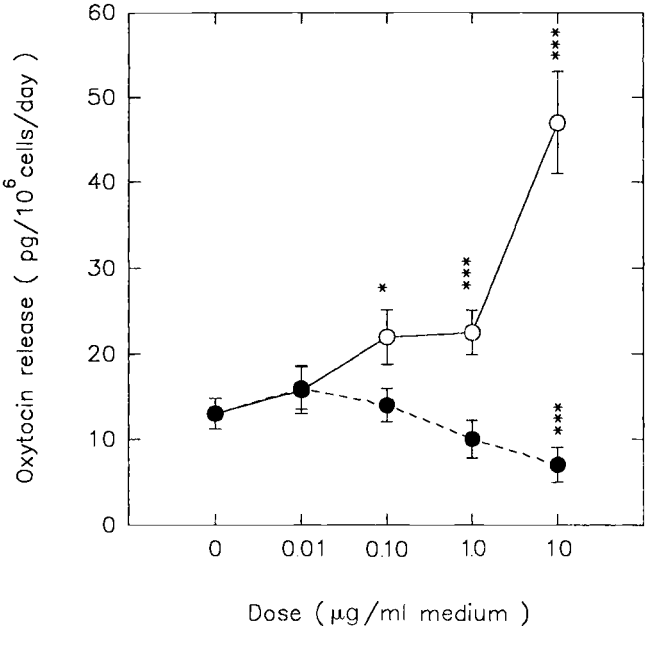

Fig. 2 Effects of melatonin (———) and serotonin (– – –) on oxytocin release by cultured human granulosa cells. Details as in Fig. 1

Tamanini, 1992; Tanaka et al., 1993). In these cases, the indoleamines tended to stimulate progestagen secretion by animal and human granulosa cells. On the other hand, the present results agree with our previous observation (Sirotkin, 1994) of a progesterone-inhibiting effect of melatonin on porcine granulosa cells. These differences may be explained by a difference in material used: all the authors cited above isolated cells from large, preovulatory follicles, while in our experiments small and medium-sized follicles were used. Although all authors report the influence of indoleamines on ovarian steroidogenesis, it is possible that the role of indoleamines in the regulation of ovarian cell function changes during follicular growth and maturation.

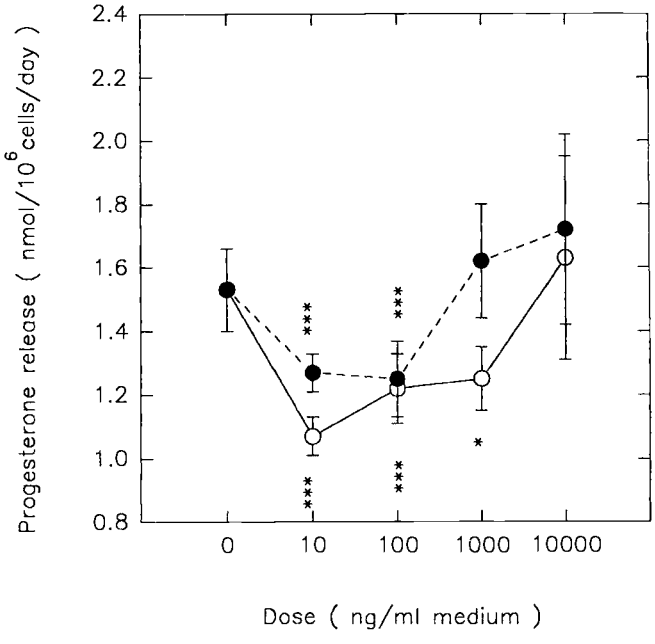

Fig. 3 Effects of melatonin (———) and serotonin (– – –) on progesterone release by cultured human granulosa cells. Details as in Fig. 1

Our data represent the first evidence for a effect of indoleamines on human ovarian non-steroidal secretion. It confirms our previous data on the influence of melatonin on the secretion of oxytocin and vasopressin by porcine granulosa cells (Sirotkin, 1994). Nonapeptide hormones are potent regulators of ovarian steroidogenesis, Corpus luteum development and reproductive tract function (Wathes, 1989). Consequently, an involvement of indoleamines in the control of these processes via ovarian oxytocin might be hypothesised.

IGF-I is known to be an important regulator of ovarian cell proliferation, steroid secretion and oocyte maturation (Adashi et al.1985; Guidice, 1992). The stimulatory influence of both melatonin and 5-HT on IGF-I in our experiments suggest a contribution of indoleamines to the control of growth factor systems. Since IGF-I from bovine granulosa cells is also able to stimulate oxytocin secretion (Schams et al.,1988; McArdle and Holtorf, 1989; McArdle et al.,1991), it

is possible that the dramatic augmentation of oxytocin resulting from melatonin addition in our experiments was due to the indoleamine-induced IGF-I output.

Thus, in conclusion, our observations suggest the involvement of both serotonin and melatonin in the control of secretion of human ovarian steroids, nonapeptide hormones and growth factors. These in turn may regulate the production of other substances within the reproductive system, including ovarian and oviduct cyclicity and oocyte maturation (see above). Although the interrelationships between mediators of indoleamine action remain to be studied, a role for circulating indoleamines in the control of human reproduction may be hypothesized.

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