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APPLICATION OF PROTEIN KINASE A STIMULATORS FOR CONTROL AND IMPROVEMENT OF REPRODUCTIVE FUNCTIONS

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ABSTRACT

In our short review we present available data concerning the involvement of regulators of cAMP and cAMP-dependent protein kinase A (PKA) in control of ovarian functions and its application in female reproduction. Activators of PKA are able to control proliferation, apoptosis and secretory activity of ovarian cells, as well as oocyte maturation. Changes in PKA induced alterations in mice and rat ovulation and fertility rate. Finally, administration of 3-isobutyl-1-methyl-xanthine (IBMX), an inhibitor of cAMP and cGMP phosphodiesterases and of dbcAMP, a cAMP analogue, led to increase of the rabbit ovarian mass, number of ovulations and *corpora lutea*, ovulated oocytes and developed embryos. These treatments changed the release of ovarian steroid hormones in vivo and in-vitro, as well as altered the expression of markers of proliferation and apoptosis in isolated ovarian and oviductal cells. These available data demonstrate that PKA is involved in control of basic ovarian and oviductal functions (proliferation, apoptosis, release of hormones, ovarian follicle development and ovulation and embryo production), and that pharmacological regulators of PKA can be practically used for stimulation of fecundity and for treatment of animal and human reproductive disorders.

Key words: rabbit; ovulation; embryo development; 3-isobutyl-1-methyl-xanthine (IBMX); N^6,2'-dibutyryladenosine 3'5'-cyclic monophosphate (dbcAMP); protein kinase A; proliferation; apoptosis

Protein kinases

Protein kinases (PKs) are enzymes catalyzing protein phosphorylation. Such phosphorylation results in a functional change of the substrate protein by changing its biological activity, cellular location, association and other interrelationships with other proteins. All PKs remove a phosphate group from ATP and covalently attach it to one of four amino acids that have a free hydroxyl group - serine and threonine (superfamily of serine-treonine PKs), tyrosine (superfamily of tyrosine kinases, TK), serine, threonine and tyrosine (dual-specificity kinases) and histidine (histidine kinases). Within these superfamilies there are sub-divisions of PKs based on their regulators. The most known representative of serine/threonine PKs are PKs regulated to cyclic adenosine monophosphate cAMP (protein kinase A, PKA) and cyclic guanosine monophosphate cGMP (protein kinase G, PKG). The PKs phosphorylate specific target proteins, which are often enzymes (other PKs, transcription factors a.o.) themselves. Therefore, single PKs or their cascades play an important role in intracellular transduction of signals from extracellular factors to target genes involved in control of the cell cycle, apoptosis, differentiation, and response to external stimuli. Some external factors (metabolism, growth factors, hormones) can exert their effects via different PKs and PK cascades (Conti, 2002; Dupont et al., 2008).

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Involvement of protein kinase A and related substances in control of reproduction

Presence of different PKs in gonads, as well as observations of effects of activators and blockers of PKs indicate presence and importance of a number of other PKs in control of ovarian functions and in mediating effects of hormones on these functions. For example, blockade of 88 PKs by siRNA constructs altered ovarian cell functions (Sirotkin et al., 2010).

The involvement of cAMP, cGMP and their target PKs, PKA and PKG, in control of reproductive processes is well documented (Dupont et al., 2008). Both cAMP and its target, cAMP-dependent PKA (Makarevich and Sirotkin, 2000; Conti, 2002; Mehlmann, 2005; Sirotkin, 2005; Hunzicker-Dunn and Maizels, 2006; Sirotkin et al., 2010) and cGMP and its target, cGMP-dependent PKG (Sirotkin et al., 2000, 2010; LaPolt et al., 2003) play an important role in control of ovarian cell proliferation, apoptosis, secretory activity and oocyte maturation.

cAMP/PKA can either promote (Makarevich and Sirotkin, 2000; Hunzicker-Dunn and Maizels, 2006) or not influence (Dupont et al., 2008) proliferation of ovarian cells. cAMP/PKA can either up-regulate (Amsterdam et al., 2003), down-regulate (Viegas et al., 2008) or not affect (Sirotkin and Makarevich, 1999; Sirotkin, 2005) ovarian cell apoptosis. cAMP/PKA in rodents and, probably in humans, can either promote (Dupont et al., 2008) or suppress (Deckel, 2005) oocyte maturation, but in porcine and bovine oocytes it prevents (Dupont et al., 2008) meiosis reinitiation.

Pharmacological regulators of PKA were able to affect ovarian steroidogenesis (release of progestagen, androgen and estrogen) (Makarevich and Sirotkin, 2000; Conti, 2002; Dupont et al., 2008). Furthermore, cAMP/PKA axis can be the key intracellular mediator of effect of upstream physiological hormonal stimulators – gonadotropins (Hunzicker-Dunn and Maizels, 2006) and GH (Makarevich and Sirotkin, 2000; Sirotkin, 2005) on these processes. Both cAMP and cGMP are hydrolysed by the enzymes - phosphodiesterases (PDEs). Mice deficient in cAMP-specific phosphodiesterases - PDE3A and PDE4, have impaired differentiation of ovarian cells, their response to gonadotropin, oocyte maturation, ovulation and fertility (Conti, 2002; Park et al., 2003; Masciarelli et al., 2004). On the other hand, synthetic PDE4 inhibitors increased cAMP accumulation in ovarian cells, as well as number of ovulations, embryos and born pups in gonadotropin-stimulated rats (McKenna et al., 2005). It is therefore possible, that PKA activators (PDE inhibitors or cAMP analogues) could be used for control of ovarian cell functions and fertility. Mechanisms of PKA action on these processes are not completely elucidated, but it might be proposed, that it can affect proliferation, apoptosis and secretory activity of ovarian and/or oviductal cells.

Effect of protein kinase A activators on rabbit reproductive processes

To examine the influence of PKA activators on farm animal reproduction and to detect its possible mechanisms, we have started a study to examine the influence of two activators of PKA, 3-isobutyl-1-methyl-xanthine (IBMX), an inhibitor of cAMP and cGMP phosphodiesterases, and a cAMP analogue, N^6,2'-dibutyryladenosine 3'5'-cyclic monophosphate (dbcAMP), on the release of hormones, expression of markers of proliferation and apoptosis in ovarian and oviductal cells, as well as on fertility in rabbits, whose ovarian cycle and ovulation were induced by gonadotropins. Part of the obtained data was present in the corresponding publications (Sirotkin et al., 2008, 2009). In our experiments, injections of IBMX significantly increased the number of ovulations, ovulated oocytes and developing embryos. Administration of IBMX reduced blood level of P_4, but did not affect blood E_2. Fragments of ovaries isolated from rabbits treated with IBMX released more E_2, but not P_4, than ovarian cells isolated from the control animals. IBMX injections substantially decreased the expression of the upper (23kD) fraction of bax. IBMX administration did not affect PCNA, but it caused a decrease in the upper fraction (54kD) and an increase in the bottom fraction (55 kD) of cyclin B1. Oviductal cells isolated from the IBMX-treated animals, contained less marker of apoptosis - bax (but not bcl-2) and proliferation - ERK1,2-related MAP kinase (but not PCNA) than control animals. Administration of dbcAMP increased the ovarian mass, number of c*orpora lutea*, number of harvested oocytes, zygotes and embryos at the blastocyst stage derived from these zygotes after culture. Administration of dbcAMP resulted in the reduction of E_2, but not P_4 level in rabbit plasma, as well as P_4 and E_2 release by isolated ovarian fragments. The additions of dbcAMP to culture medium promoted accumulation of both proliferation and apoptosis markers in cultured ovarian fragments. These data demonstrate that IBMX and dbcAMP can enhance the stimulatory effect of gonadotropins on the rabbit ovarian and oviductal functions and on rabbit fecundity via changes in ovarian hormone release, ovarian and oviductal cell proliferation and apoptosis.

Our observations confirm the data obtained on rats (McKenna et al., 2005) and mice (Conti, 2002; Park et al., 2003; Masciarelli et al., 2004), that cAMP/PKA could be stimulators of fertility, which could, in addition to- and even instead of gonadotropins, promote rodent ovarian folliculogenesis, ovulation, oocyte maturation, fertility and embryo production. Present observations are the first demonstration of the involvement of PKA in control of rabbit reproductive processes *in vivo*. These findings are in line with previous reports on the involvement of cAMP (Makarevich and Sirotkin, 2000; Conti, 2002;

Mehlmann, 2005; Sirotkin, 2005; Hunzicker-Dunn and Maizels, 2006), cGMP (Sirotkin et al., 2000; LaPolt et al., 2003) and their downstream signaling pathways in control of ovarian cell functions.

The data concerning the influence of both IBMX and dbcAMP on basic functions of ovarian and oviduct cells suggest, that these activators of PKA can promote reproductive functions through stimulation of proliferation, inhibition of apoptosis, and/or changes in ovarian and oviduct cell turnover. Furthermore, the stimulatory effect of PKA stimulators could be due to changes in production, secretion or metabolism of ovarian steroid hormones - P_4 and E_2. Finally, PKA stimulators can potentially not only affect basic reproductive functions, but also enhance the response of rabbit reproductive system to gonadotropins.

CONCLUSION

Our observations, together with previous publications, demonstrate an important role of cAMP/PKA-dependent signaling pathway in control of basic reproductive functions (proliferation, apoptosis, gametogenesis and secretory activity). Furthermore, they suggest, that pharmacological stimulators of PKA could be used for the improvement of gonadotropin action on basic reproductive functions. They could be potent alternative stimulators of fertility in animal and human assisted reproduction and biotechnology, as well as for the treatment of reproductive disorders (involving ovarian insufficiency) in human and veterinary medicine.

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