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Oxytocin as an Inducer of Cardiomyogenesis

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1. Introduction

The first description of the uterotonic action of pituitary extracts was first described by Sir Henry Dale in 1906 (Dale, 1906). A few years later, Ott and Scott demonstrated that besides the effect on uterine activity, posterior pituitary extracts also promoted milk ejection (Ott & Scott, 1910). These are the 2 principal activities of oxytocin (OT), the structure and synthesis of which were not elucidated until 50 years later by Du Vigneaud and co-workers (Du Vigneaud, 1956). OT is mainly produced in the paraventricular nucleus and supraoptic nucleus of the hypothalamus, and released from hypothalamic nerve terminals of the posterior pituitary, where it is stored, into the bloodstreem. OT differs, by only two amino acids, from vasopressin (AVP), which is also produced in these nuclei and stored in the posterior pituitary. It was previously believed that OT as well as AVP were exclusively released from the neurohypophysis although Ott and Scott (Ott & Scott, 1910) reported that the extracts of other tissues such as the corpus luteum, pineal and thymus glands have the same milk-ejecting properties. Early studies associated OT with cardiovascular system Paton and Watson first described the blood pressure (BP) depressor response to posterior pituitary extract in avians (Paton & Watson, 1912). Hogben & Schlapp confirmed their findings and showed the effect of whole posterior pituitary extracts in amphibians and reptiles (Hogben & Schlapp, 1924). Furthermore, this effect was evoked by histamine-free extracts and was therefore a pituitary action. When separated fractions of posterior pituitary extracts obtained by fractional precipitation became available, Gaddum showed that the depressor response was attributable to the only one fraction, called oxytocin, which had oxytocic properties (Gaddum, 1928). These observations were completely overlooked.
However, recent studies have shown that OT is an ubiquitous hormone, synthesized at many locis, and a wide array of physiological activities has been attributed to this peptide. Similar numbers of oxytocinergic neurons have been found in the male and female hypothalamus, and the same stimuli induce OT release in both genders, suggesting other physiological functions that its role in female reproduction. In fact, OT receptors (OTR) are also widely expressed in diverse tissues such as the pituitary, kidney, ovary, testis, thymus, heart, vascular endothelium, osteoclasts, myoblasts, pancreatic islets, adipocytes and several types of cancer cells (Gimpl & Fahrenholz, 2001). OT elicits a variety of physiological responses such as complex sexual and maternal behavior. Indeed, OT is also involved in

cognition, tolerance and cardiovascular regulation. OT acts on one type of OTR, an integral membrane protein that is a member of the rhodopsin-type (class I) G protein-coupled receptor family, which includes AVP receptor subtypes (V1aR, V1bR and V2).

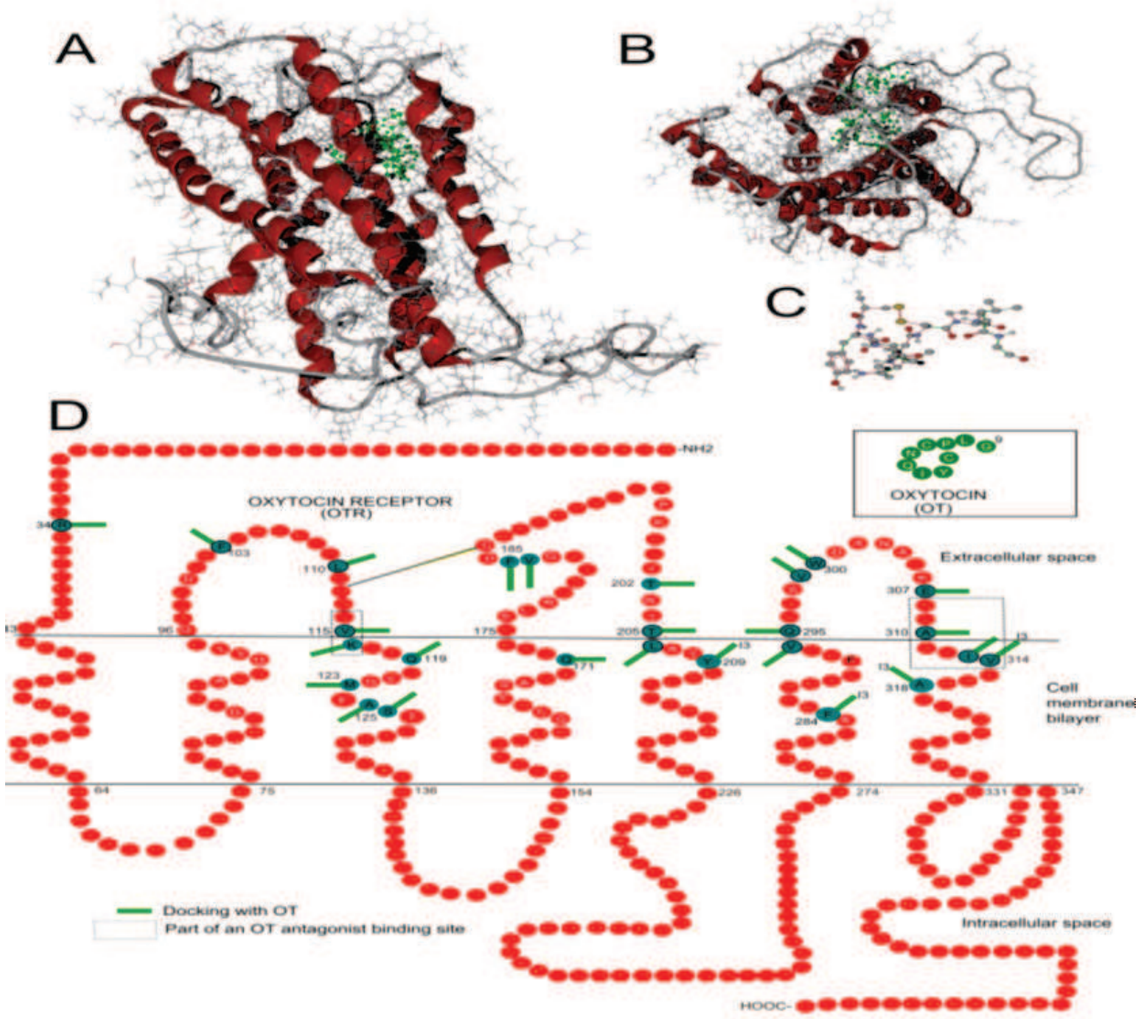

Fig. 1. Several oxytocin binding sites are present on oxytocin receptor. This figure illustrates molecular docking of the three-dimensional models of activated human oxytocin receptor with oxytocin obtained by MolDock Optimizer algorithm from Molegro Virtual Docker software. (A) The front upright view position (side view) of the receptor structure with oxytocin. (B) Panel B shows an intracellular view (i.e. rotation by 90º out of plane). (C) Conformational view of oxytocin molecule. (D) The schematic model of the human oxytocin receptor with marked amino-acid residues that are putatively involved in ligand-binding

All of these receptors have the ability to bind AVP and/or OT with different affinities. Because OT system activation was observed in fetal and newborn hearts at a stage of intense cardiac hyperplasia (Jankowski et al., 2004), we hypothesized a role for OT in cardiomyocyte (CM) differentiation. The initial experiments demonstrated that OT induces CM differentiation of the mouse embryonal carcinoma (EC) P19 cell line, a common stem cell model for studying early heart differentiation (Paquin et al., 2002). Then, several studies

attempted to understand the mechanisms of cardiac differentiation by OT. Stimulation of CM differentiation can be concomitant with neovascularization because OT stimulates endothelial cells growth and angiogenesis. In pathological conditions such as cardiac ischemia and diabetes this inducer can be used to stimulate production of lost cardiac cells. The advantage of this therapy is supported by the fact that OT is endogenously produced in the organism, and does not have significant side effects when used in clinics. Moreover, it is now possible to inject (transplant) one's own stem cells after previous stimulation with OT inducers, as in the case of a heart infarct. Alternatively, direct treatment with OT molecules could promote cardiomyogenesis in situ and regeneration of a damaged heart.

2. OT induces stem cell differentiation

The manipulations of OT, during the perinatal period have become an accepted—but largely unstudied—aspect of human development. For example, synthetic OT is used to induce childbirth (Dudley, 1997). In cases of premature delivery, the OT antagonist (OTA) is used for slowing or preventing labor (Husslein, 2002). Although such complex manipulations are routine in modern obstetrics, little is known on the possible cellular and developmental consequences of these treatments. Several studies have proposed a role for OT as a growth and differentiation/maturation factor in a gestational/perinatal context. In the mother, OT is required for postpartum alveolar proliferation, and it induces differentiation and proliferation of myoepithelial cells in the mammary gland necessary for milk ejection (Gimpl & Fahrenholz, 2001). The OT/OTR system is expressed in human cumulus/luteal cells surrounding oocytes, and weak OTR gene expression is even observed in oocytes (Furuya et al., 1995). Moreover, when fertilized mouse oocytes are cultured with OT *in vitro*, they develop into the blastocyst stage at a higher rate than their unstimulated counterparts (Furuya, et al., 1995).

On the other hand, hormonal treatment may, in its simplest form, induce mammalian stem cells into a special cell type that retains the ability to self-renew (i.e. undergo cell division in an undifferentiated state) indefinitely and to differentiate into specialized cells. In this regard, it has been suggested that OT plays a role in bone homeostasis and osteoporosis based on the proliferative effects of OT on osteoblasts in vitro and the modulation of blood parameters associated with bone formation in normal rats (Petersson et al., 2002b). Recently, OT has been implicated in the regulation of the osteoblast/adipocyte balance of human multipotent adipose-derived stem cells and human bone marrow mesenchymal stromal cells (Elabd et al., 2008).

Our interest in the cardiac OT system emerged from long time investigations into the role of the brain in the control of cardio-renal homeostasis (Gutkowska & Jankowski, 2008; Gutkowska et al., 2000; McCann et al., 2002). These experiments led to the observations that OT and its receptor (OTR) are synthesized in the human and rat heart (Gutkowska et al., 1997; Jankowski et al., 1998) and that OT exerts cardio-protection either directly or via stimulation of mediators such as the natriuretic peptides (NPs) (Gutkowska et al., 1997; Gutkowska et al., 2000) and nitric oxide (NO) (Danalache et al., 2007). Since the study showing that OT induces differentiation of EC P19 cells into the functional cardiac muscle (Paquin et al., 2002), several reports have confirmed OT-stimulated cardiomyogenesis in different lines of embryonic stem cells (Fathi et al., 2009; Gassanov et al., 2008a; Hatami et al., 2007; Jankowski et al., 2004; Stefanidis et al., 2009; Uchida et al., 2007). In fact, OT can be considered as an established myogenic morphogen (Breton et al., 2002). It induces

differentiation of myoepithelial cells in the mammary gland (Sapino et al., 1993). The skeletal myocytes generation has also been observed in OT-induced cardiomyogenesis (Danalache et al., 2007) as well as adipogenesis (Bouchard & Paquin, 2009). OT increases the rate of myoblast fusion and myotubule formation (Breton et al., 2001). Interestingly, most transcription factors identified so far in the heart are also present in other muscle cells, and myoblast transplantation to the injured heart improves regional systolic heart function (Thompson et al., 2003). Assuming similarities in the differentiation mechanisms of skeletal and cardiac muscles and the expression of both OT and OTR in these cells (Breton et al., 2001; Jankowski et al., 1998), we can speculate that OT plays a role in cardiac muscle regeneration.

3. OT stimulates CM differentiation in 3-dimensional cultures of stem cells

EC P19 cells are derived from a teratocarcinoma in CH3/He mice and can differentiate into all 3 germ layers (van der Heyden & Defize, 2003). Developmentally, pluripotent EC P19 cells appear to differentiate by the same mechanisms as normal embryonic stem cells (McBurney, 1993). Culture and differentiation of the cells is simple and cells remain undifferentiated without the help of feeder cells or inhibitory factors, and unlike embryonic ES cells, they do not spontaneously generate cardiomyocytes. The efficient differentiation of EC P19 cells depends on the prior formation of non-adhering cell aggregates (van der Heyden & Defize, 2003). Traditionally, cell aggregates formation in suspension culture under 0.5–1.0% dimethyl sulfoxide (DMSO) has been used to induce cardiomyocyte differentiation of EC P19 cells (McBurney, 1993). Efficiency of cardiac differentiation of ES and EC P19 cells in vitro is still not optimal in response to various agents, with yields varying between 5% and 20% of cardiomyocytes (Danalache et al., 2007; Gassanov et al., 2008a; Gassanov et al., 2008b; Gassanov et al., 2007; Paquin et al., 2002). In the EC P19 model, the order of cardiomyogenesis efficiency was OT (10^{-7} M) ≥ DMSO >retinoic acid (10^{-8}–10^{-7} M) when these agents were added to cultures during the entire period of cell aggregation (Danalache et al., 2007; Jankowski et al., 2004; Paquin et al., 2002). It is noteworthy that exposure of EC P19 cells to higher retinoic acid concentration (10^{-6} M) over the aggregation period generates neurons but not muscle cells.

The presence of OT significantly influences the shape and size of aggregated stem cells isolated from the rat heart (Gutkowska & Jankowski, 2009). This suggests that conditions inside aggregates, such as hypoxia, promote OTR and OT expression. Indeed, hypoxia can influence the functionality of OTR in cardiac cells and mechanism of natriuretic peptides secretion in response to OT treatments (Hopkins et al., 2004). Our data indicate that OT increases glucose uptake by CMs exposed to chemical hypoxia (Florian et al., 2010). Multi-cellular complex aggregate formation and exposure to various agents promotes generation of mesodermal or ectodermal lineages. In embryonic D3 stem cells, spontaneously producing beating cell colonies upon aggregation, the mesodermal derivatives formed in embryoid bodies (EB) include subtypes of cardiac cells (atrial CMs, ventricular CMs and pacemaker cells) which are potently enhanced by treatment with OT as identified by histological, molecular, and electrophysiological criteria (Gassanov et al., 2008a). However, molecular events occurring during aggregation and the necessity of their aggregation for differentiation are not entirely understood. High cell densities can at least trigger spontaneous differentiation from EC P19 cells (McBurney, 1993). Skerjanc et al. have reported that overexpression of Nkx2.5 can induce cardiomyogenesis in aggregated EC P19

cells, but not when they are maintained in monolayer cultures (Skerjanc et al., 1998). On the other hand, the addition of soluble bone morphogenic protein 4 (BMP 4) into the culture media resulted in the requirement for cell aggregation and induced cardiac differentiation being bypassed in monolayer cultures of EC P19 cells overexpressing Nkx2.5 (Jamali et al., 2001). This result demonstrated that cell aggregation is crucial for the generation of the BMP 4 signal in EC P19 cells. EC P19Cl6 cells derived from EC P19 cells seem not to be committed to a mesodermal lineage but rather represent a stage closer to differentiated cardiac muscle than the parental cell line. It was recently observed that OT does not induce cardiomyogenesis in monolayers of EC P19Cl6 cells, but does so in aggregates (Uchida et al., 2007). The same authors suggested that OT-induced cardiac differentiation is not mediated by the expression of BMP 4 signaling molecules because the induction of BMP 4 signaling cascade can bypass the requirement for prior EB formation (Fathi et al., 2009).

4. OT molecular forms of OT stimulating differentiation

OT, recognized as a female reproductive hormone, is largely produced in hypothalamic magnocellular neurons of paraventricular and supraoptic nuclei. Biochemical and recombinant DNA studies have demonstrated that it is synthesized as a non-glycosylated protein, which undergoes an initial endoproteolytic cleavage by the convertase magnolysin (EC 3.4.24.62) to OT-Gly-Lys-Arg (OT-GKR) (Brownstein et al., 1980; Burbach et al., 2001). Subsequent processing produces other OT extended molecules: OT-Gly-Lys (OT-GK) and OT-Gly (OT-G) (Burbach et al., 2001), all these forms are often referred to as OT-X (Morris et al., 1992). OT-G is converted by an α-amidating enzyme to C-amidated nonapeptide. OT is released into the bloodstream in this form. OT-X forms have been detected in the developing brain of animals and in fetal plasma. In rats, enzymatic OT-X conversion to OT is almost complete in adulthood, but not in fetuses, which accumulate OT-X in the brain (Altstein et al., 1988a; Altstein & Gainer, 1988b. Similarly, the plasma OT-X elevation reported during early fetal development in sheep (Morris et al., 1992) is reduced in late gestation, when OT begins to predominate in the cbloodstream.

We have recently shown that the OT-GKR is the main form in the fetal heart. This attracted our attention because estrogen-mediated BP reduction in humans is associated with elevated plasma OT-GKR but not OT, indicating greater bioactivity and stability of this form (Light et al., 2005). We have also found that OT-GKR even more potently than OT mediates cardiomyogenesis in EC P19 cells (Jankowski et al., 2004) and D3 stem cells (Gassanov et al., 2008a). The patch-clamp analysis indicates that OT-GKR differentiates D3 cells into CMs of the ventricular phenotype and reduces formation of pacemaker cells (Gassanov et al., 2008a). Furthermore, OT-GKR significantly increases glucose uptake in CM exposed to hypoxia (Florian et al., 2010).

To determine whether the genetic modification of stem cells also stimulates cardiomyogenesis, the OT-Gly-Lys-Arg gene was inserted into D3 stem cells. In effect, we observed stimulation of spontaneously-beating embryoid bodies and predominant stimulation of cells expressing the ventricular electrophenotype and molecular CM markers (Gassanov et al., 2008a). Interestingly, the elongated form of OT, OT-Gly-Lys-Arg, was the most potent cardiomyogen of all the OT-like molecules investigated. These findings provide a new strategy for the regeneration of diseased hearts. Transgenic cells producing OT-Gly-Lys-Arg can be injected, for cardioprotection, into the myocardial infarcted rat model. Analysis of cardiac remodeling, scar reduction, hemodynamic and echographic parameters

together with histochemical and molecular analyses will provide answers as to whether these treatments can stimulate cardioprotection.

5. Mechanism in OT-induced stem cell differentiation

Some preliminary observations point to a mechanism involved in this process. Ca^{+2} mobilization in response to OT treatment has been detected in D3 ES cells differentiating into CMs (Gassanov et al., 2008a). It has also been shown that OT-induced differentiation of EC P19 stem cells into CMs is inhibited by the NOS inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME).

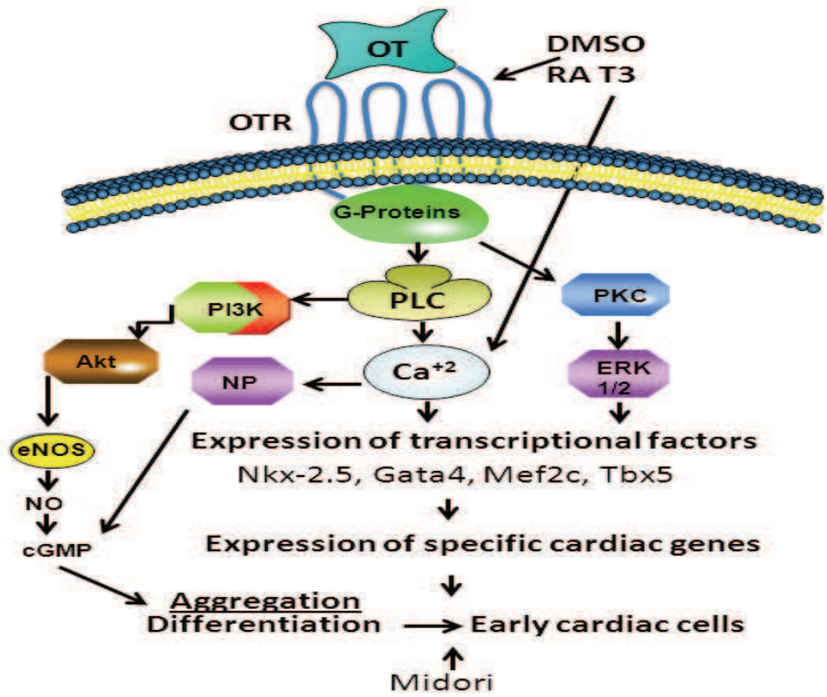

Fig. 2. Signal transduction cascades involved in oxytocin-mediated cardiac differentiation of P19 embryonal carcinoma cells. See text for details. Akt indicates protein kinase B; ERK, extracellular signal-regulated kinase; eNOS, endothelial nitric oxide synthase; midori, (myocyte induction/differentiator originator); NP, natriuretic peptides; PI3K, phosphatidylinositol 3-kinase; PLC, phospolipase C; PKC, phosphokinase C, RA, retinoic acid

The NO donor *S*-nitroso-*N*-acetylpenicillamine (SNAP) was able to reverse L-NAME-mediated inhibition of EC P19 cell differentiation into CMs (Danalache et al., 2007). This study clearly indicates a role for NO and NOS enzymes in stem cell differentiation, but it is evident that thjis may be a complex process. This complexity is highlighted by the fact that suppression of NOS activity by L-NAME has also been shown to increase the number of stem and progenitor cells differentiating into CMs (Danalache et al., 2007). Another study has reported that exposure of D3 stem cells to AVP increases the number of beating

embryoid bodies and also heightens GATA-4 expression. These AVP effects on the cells were also found to be antagonized by L-NAME (Gassanov et al., 2007), again suggesting a positive role for NO in stem cell differentiation into CMs. This investigation highlighted the expression of AVP receptors in undifferentiated D3 cells, with the expression profile changing during the differentiation process (Gassanov et al., 2007). It has been observed in the EC P19 cell model that AVP not only increases spontaneously-occurring cardiomyogenesis but also initiates the process (Gassanov et al., 2007; Gutkowska et al., 2007).

The OTR-NO-cGMP pathway that is essential for OT-elicited differentiation of EC P19 stem cells into CMs is associated with elevation of GATA-4 and myocyte enhancer factor 2c (MEF2c) (Danalache et al., 2007). GATA-4 regulates the expression of genes that are critical for CM differentiation. MEF2c is a member of the MEF2 family that is involved in cardiac, skeletal, and smooth muscle development. Partial GATA-4 gene targeting in cardiac and non-cardiac cells indicates that even modest variations in GATA-4 gene level or activity can play a role in the maintenance of normal cardiac function (Bisping et al., 2006). GATA-4 has also been implicated in intercellular cross-talk by inducing hypertrophy-associated angiogenesis via vascular endothelial growth factor (VEGF) release and targeting the endothelium (Heineke et al., 2007). The upstream sequence of OTR contains putative binding sites for GATA-4 and Nkx2.5 and GATA-4 also serves as a key transcriptional regulator of numerous cardiac peptides, including ANP, BNP and OTR (Uchida et al., 2007). GATA-4 has also been identified in stem and progenitor cells of the heart in combination with OT-mediated CM differentiation (Matsuura et al., 2004; Oyama et al., 2007). A recent study has demonstrated that undifferentiated murine ES cells express BNP and its receptors, with its signaling being essential for cell survival and clonal growth (Abdelalim & Tooyama, 2009). This observation suggests possible interaction of the OT and NP systems in ES cells during cardiomyogenesis.

6. Differentiation of endogenous stem cells isolated from animal hearts

The idea that OT has cardio-regenerative capacities is supported by the observation that this hormone induces the differentiation of cultured mice (Matsuura et al., 2004) and rats (Oyama et al., 2007) resident cardiac stem cells (CSCs). In the adult heart, CSCs maintain a balance of survival, proliferation and self-renewal to replace mature cells that are lost during injury or turnover. Matsuura's group revealed the presence of a Sca-1$^+$ stem cell population in adult mouse hearts expressing telomerase reverse transcriptase, which has been associated with self-renewal potential (Matsuura et al., 2004). These cells, lacking hematopoietic markers, are easily distinguished from hematopoietic stem cells of bone marrow origin, and when treated with OT, differentiate into functional CMs. Although the cells present the early cardiac markers GATA-4 and MEF2, they do not express Nkx-2.5 or genes encoding cardiac sarcomeric proteins. When exposed to OT, a small population of Sca-1$^+$ cells manifest sarcomeric structures and form spontaneously-beating CMs. In addition, after intravenous delivery, Sca-1+ cardiac stem cells can be recruited to the myocardium injured by ischemia/reperfusion and can functionally differentiate *in situ* (Matsuura et al., 2004). Importantly, these cells had positive ionotropic responses to isoproterenol via β1-adrenergic receptor signaling. Given the apparently small number of CMs generated in vitro by OT stimulation, this raises the questionof whether or not OT-mediated cardiomyogenesis is a default pathway for CSCs. Accordingly, Matsuura et al.

reported that OT induces about 0.5-1% of Sca-1^{POS} ckit^{NEG} CD45^{NEG} cells from the adult murine heart to differentiate into functional, spontaneously-beating immature CMs (Matsuura, et al., 2004). In this regard, the cardiac differentiation of Sca-1+ cells does not require cell aggregation for the process to proceed (Matsuura, et al., 2004). On the other hand, a study by the same group in another (CSC) type isolated from the rat heart (Oyama, et al., 2007) disclosed that OT treatment resulted in the generation of 5% CMs. These cells, termed cardiac side population cells (CSPs), but not to corresponding side population cells isolated from bone marrow, differentiated into CMs in response to OT treatment. Therefore, OT possesses more powerful cardiogenic activity against CSCs than previously reported. CSPs have the ability to efflux Hoechst dye, a process dependent on ABC transporters. CSCs, especially Abcg2-dependent CSPs, have been associated with stem/progenitor cells. These cells are positive for Abcg2, Sca-1, ckit (low), CD34 (low), CD45 (low) and negative for CD31 (Mouquet et al., 2005; Pfister et al., 2005). A possible role for CSCs in heart healing is indicated by increased numbers of Abcg2-expressing cells in the border zone adjacent to myocardial infarcts (Pfister, et al., 2005). Stimulation of CM differentiation could be concomitant with neovascularization because OT stimulates endothelial cell growth (Thibonnier et al., 1999) and angiogenesis (Cattaneo et al., 2008).

7. OT mediates cardioprotection

The absence of either OT or its receptors in knockout mice, however, has not been reported to produce cardiac insufficiencies (Nishimori et al., 2008; Takayanagi et al., 2008). Although OT knockout mice have a normal heart structure, experiments have shown augmented intrinsic heart rates in these animals, indicating that an intracardiac OT system controls cardiac electrical activity (Bernatova et al., 2003).

Although the pathophysiological role of OT is beginning to be understood, accumulating evidence indicates multiple beneficial effects in the heart and vasculature. To date, OT's cardiovascular properties include: i. natriuresis (Soares et al., 1999) and decreased blood pressure (BP), possibly secondary to atrial natriuretic peptide (ANP) release (Gutkowska et al., 1997; Petersson, 2002a) ii. negative inotropic and chronotropic effects (Favaretto et al., 1997; Ondrejcakova et al., 2009) and parasympathetic neuromodulation (Mukaddam-Daher et al., 2001); iii. vasodilatation via the OTR-induced NO pathway; iv. endothelial cell growth and possible vessel generation (Cattaneo et al., 2008; Cattaneo et al., 2009; Thibonnier et al., 1999); and v. modulation of insulin release (Sirotkin et al., 2003) and anti-diabetic actions (Florian, et al., 2010). At the cellular level, protective OT: i. has antioxidant properties (Iseri et al., 2005a, 2005b) ii. has anti-inflammatory actions (Jankowski et al., 2010a; Szeto et al., 2008), iii. potentiates glucose uptake in neonatal and adult CMs exposed to hypoxia and conditions of insulin resistance mimicked by the presence of ketone bodies (Florian, et al., 2010) iv. stimulates endothelial markers in mesenchymal cells (Kim et al., 2010) and stem cells isolated from the heart as a side population (Oyama et al., 2007).

Central, intraventricular infusion of OT is accompanied by an increase in blood pressure; this effect is probably associated with the stimulation of substance P forebrain receptors by OT (McCann et al., 2002). OT's ability to raise blood pressure is caused not only by its vasoconstrictory activity but also by antidiuretic activity. Peripheral administration of OT, on the contrary, lowers the average arterial pressure in rats and does not affect heart rate (Petersson, 2002a). On the other hand, in the absence of a central regulatory influence, OT can bring down the heart rate and reduce the strength of contractions of isolated atria

during perfusion of rat hearts (Favaretto et al., 1997; Mukaddam-Daher et al., 2001). In addition, intracardiac OT stimulating the release of ANP may control cardiovascular homeostasis and the body's internal environment (Favaretto et al., 1997; Gutkowska et al., 1997).

OT's negative chronotropic action was recently associated with attenuation of cardiac damage evinced by ischemia-reperfusion (Ondrejcakova et al., 2009). Positive cardiac effects can also be attributed to the fact that OT stimulates ANP release (Gutkowska et al., 1997) by improving hydromineral homeostasis as well as cardiac hypertrophy and reducing pro-inflammatory mediators (Jankowski et al., 2010a). ANP, a member of the NPs family that includes BNP, C type natriuretic peptide and urodilatin, is released into the circulation after volume expansion, atrial stretch (Dietz, 2005), hypoxia (Toth et al., 1994) and in response to various hormones and neurotransmitters (Antunes-Rodrigues et al., 1997; McCann et al., 2002). ANP causes BP to decline with a concomitant increment of diuresis, natriuresis and decrease plasma volume (Christensen, 1993; Ruskoaho, 1992). NPs also inhibit the sympathetic nervous system and hormones involved in cardiac hypertrophy, such as angiotensin II, endothelin and AVP (Gerstberger et al., 1992; Jankowski, 2009; Kaneko et al., 1988; Mukaddam-Daher et al., 2009; Neuser et al., 993). NPs signaling via functional receptors (NPR-A and NPR-B) prevents pathological hypertrophy (Oliver et al., 1997) and cardiac fibrosis (Calderone et al., 1998) by attenuating both DNA and collagen synthesis in cardiac fibroblasts, oxidative stress (Baldini et al., 2005; De Vito et al., 2003) and inflammation (Kiemer & Vollmar, 2001). Recent reports indicate that BNP and ANP activity is associated with lipolysis and postprandial lipid oxidation (Birkenfeld et al., 2008). Both hormones modulate fatty acid trafficking and prevent triglyceride accumulation in CMs via cGMP signaling (Khairallah et al., 2008).

The different cardioprotective actions of OT were recently demonstrated in animal models of myocardial infarction (MI). In rat and rabbit models of ischemic heart disease, OT treatment significantly reduced infarct size and improved parameters of heart function (Alizadeh et al., 2010; Houshmand et al., 2009; Jankowski et al., 2010a; Kim et al., 2010; Kobayashi et al., 2009; Ondrejcakova et al., 2009).

8. OT Signaling in cardiovascular system

In cardiac cells, several signaling pathways have also been postulated in conjunction with specific functions in the heart. Figure 1 illustrates the hypothetical pathways in the heart that are associated with OT-mediated cardioprotection, such as the prevention of apoptosis, CM hypertrophy and fibrosis, with stimulation of glucose uptake, cell proliferation and differentiation.

OT signaling depends on coupling to specific G-proteins, cell type and localization on the cell membrane surface. As a result, OTR stimulates different second messengers, which consequently exerts various physiological effects (Reversi et al., 2005). Due to its organ- and tissue-specific expression patterns, it is believed that OTR is regulated largely at the gene transcription level (Devost et al., 2008; Zingg & Laporte, 2003). In the cardiovascular system, OTR is associated with the ANP-cGMP and NO-cGMP pathways, which reduce the force and rate of contraction and increase vasodilatation.

Our recent report shows that OT increases glucose uptake in CMs via phosphoinositide-3-kinase (PI3K) and potentiates the glucose uptake effect of 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation targeting the mitochondria (Florian, et al., 2010). PI3K pathways

are considered beneficial during myocardial injuries (Cantley, 2002; Fujio et al., 2000; Miki et al., 2007). The calcium-calmodulin kinase kinase (Ca-CAMKK) and AMP-activated protein kinase (AMPK) pathways are also involved in OT-mediated glucose uptake in CMs (Florian et al., 2010). AMPK activation in the heart after ischemia and reperfusion is recognized as cardioprotective because AMPK limits both apoptosis and cell damage because both limits apoptosis and cell damage (Lee et al., 2008; Miki et al., 2007; Russell et al., 2004). We should also consider p38 MAPK and extracellular signal-regulated kinase 1/2 (ERK 1/2) phosphorylation which may contribute to OT's proliferative activity (Devost et al., 2008). More recently, in a rabbit model of myocardial ischemia-reperfusion, OT treatment induced ERK1/2, AKT and eNOS phosphorylation in cardiac tissues (Kobayashi et al., 2009). Therefore, OT, like other G-protein-coupled ligands, can act by PI3K/AKT activation and projection onto downstream kinases. Recent studies have demonstrated that the cardioprotective effects of OT are mediated through opening the mitochondrial ATP-dependent potassium (mitoKATP) channels in the rat heart (Alizadeh et al., 2010).

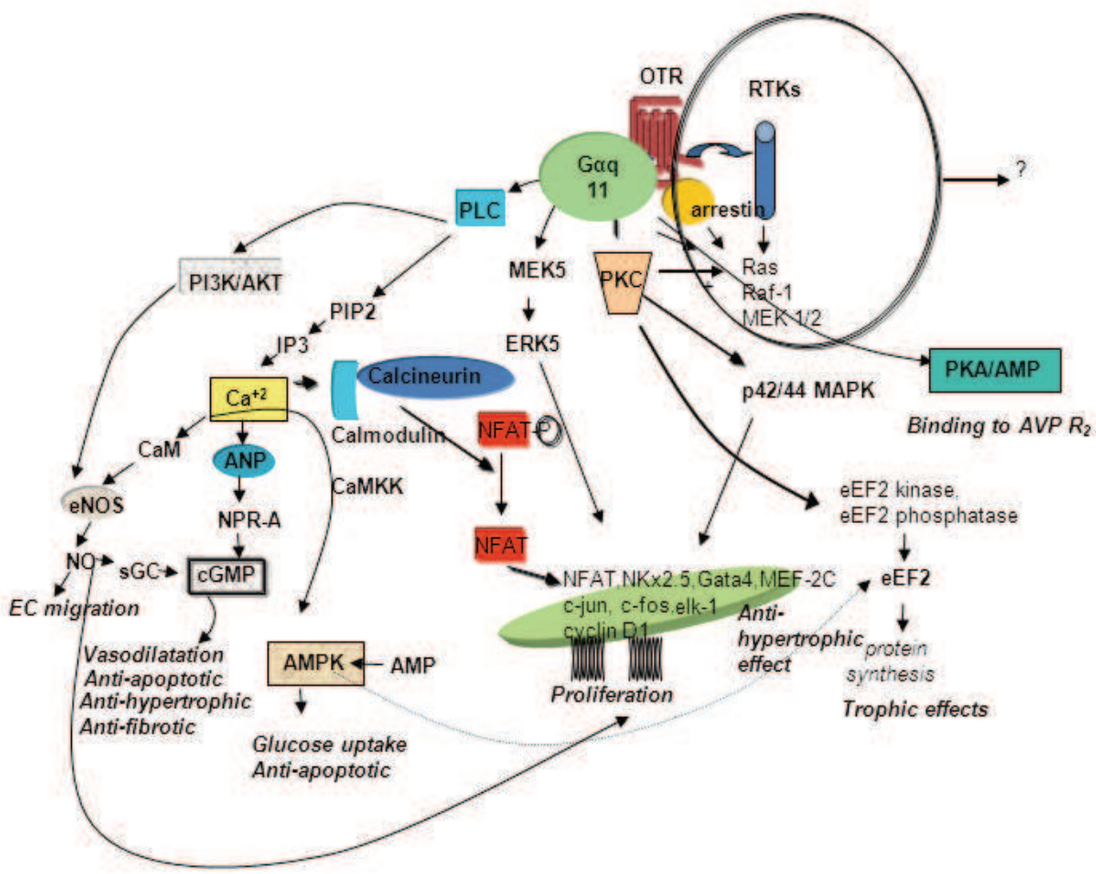

Fig. 3. Schematic diagram of potential protective pathways of oxytocin in the cardiomyocytes. AMPK, AMP activated protein kinase; ANP, atrial natriuretic peptide; CaMKK – Ca^{+2} calmodulin-dependent protein kinase; $AVPR_2$ –Vasopressin receptor R2; EC, endothelial cells; eEF2, eukaryotic translation elongation factor 2; MEK, protein kinase, mitogen activated kinase; NFAT, nuclear factor of activated T-cells

OT promotes the migration of human dermal EC, breast-derived EC (Cassoni et al., 2006) and human umbilical vein EC (HUVEC) (Cattaneo et al., 2008; Cattaneo et al., 2009). The pro-migratory effect of OT requires OTR activation of the phosphatidylinositol-3-kinase (PI3K)/AKT/eNOS pathway (Cattaneo et al., 2009). Moreover, OT increases proliferation of EC and alters gene expression for adhesion molecules and matrix metalloproteinases (MMPs), contributing to improved cell motility and growth (Cassoni et al., 2006). Angiogenic and anti-apoptotic OT effect was indicated by increased $CD31^+$ microvessels (Jankowski et al., 2010b). In this way, OT can control blood flow to the heart.

9. OT in stem cells therapy

Cardiovascular disease is one of the leading causes of death throughout the world (Jain et al., 2005). Following myocardial infarct (MI), endogenous repair mechanisms are insufficient for meaningful regeneration, therefore, muscle lost is replaced by non-contractile fibrotic scar (Laflamme et al., 2007). Because cardiomyocytes are unable to regenerate in the adult heart, cell-based therapy of transplantation provides a potential alternative approach to replace damaged myocardial tissue and restore cardiac function. A major roadblock toward this goal is the lack of donor cells, therefore, it is urgent to identify the cardiovascular cells that are necessary for achieving cardiac muscle regeneration (Dowell et al., 2003), to treat heart failure (Dowell et al., 2003; Raeburn et al., 2002) and restore function. (Hassink et al., 2003; Orlic et al., 2002). Several candidates have been investigated: fetal (Li et al., 1997) and neonatal (Watanabe et al., 1998) CM, embryonic stem cells (Min et al., 2002; Min et al., 2003), cardiac resident stem cells, and skeletal myoblasts (Dimmeler, Zeiher, & Schneider, 2005; Leor et al., 1996; Menasche et al., 2001; Menasche et al., 2003; Murry et al., 1996; Taylor et al., 1998). Alternative source are the bone marrow-derived stem cells (Dimmeler, et al., 2005). However, recent studies have questioned the ability of implanted, untreated stem cells to generate new CMs (Laflamme et al., 2007; Murry et al., 2004; Noiseux et al., 2006). Most cells die within hours of transplantation due to the interplay of ischemia, inflammation, and apoptosis (Menasche, 2009; Rosenzweig, 2006).

Among the cells used, the MSCs are more suitable for the cell therapy because of easy isolation, high expansion potential giving an unlimited pool of transplantable cells, low immunogenicity, amenability to *ex vivo* genetic modification and multipotency. It has been shown that injection of MSC either, directly into infarcted hearts (Gnecchi & Melo, 2009) by intramuscular (Shabbir et al., 2009) or intraperitoneal injections (Takahashi et al., 2006) improves myocardial function and repair. Following exposure to hypoxia and serum starvation, conditions that mimic MI, MSC are stimulated to secrete several growth factors and cytokines (Gnecchi & Melo, 2009). The exact mechanisms underlying MSC therapeutic effects require further investigations and challenges remain in optimizing the culture-expansion conditions, the MSC capacity for growth factor production and conditions to direct stem cells differentiation into CMs or endothelial cells. Recent studies have shown that direct injection with OT-treated MSCs into the rat heart after ischemia-reperfusion injury improves the engraftment rate and results in an enhanced cardio-protective effect via: increased transmigration activity, the upregulation of matrix metalloproteinase-2 mRNA, the integration of MSCs into the myocardium as well as the anti-fibrotic and anti-inflammatory effects (Kim et al., 2010).

We propose that MSCs and other types of the stem cells should be treated with OT before their engraftment *in vivo*. Based on our preliminary data, we expect that preconditioning

with OT will enhance the capacity of these cells to repair infarcted myocardium due to reduced cell death after implantation, increased angiogenic potential, and by enhanced secretion of paracrine factors. Alternatively, the therapeutical potential of these cells can be improved by the introduction of the OT-expressing construct as recently demonstrated (Gassanov et al., 2008a).

10. Summary and conclusions

Our research lead to the observation that OT and OTR are synthesized in CMs, and we have identified OT as a potent, naturally-occurring cardiomyogenic factor, which by OTR up-regulation promotes the differentiation of embryonic and somatic stem cells residing in the heart to mature and functional CMs.

All these OT actions have physiological relevance, particularly on glucose uptake in CMs, since it is reduced in hearts from insulin-resistant diabetic mice, a disturbance that culminates in cardiac dysfunction.

Understanding the mechanisms of cardiac differentiation by OT can provide therapeutic approaches to the management of heart diseases. Currently, it is still extremely difficult to obtain new cardiac cells *in vitro* using stem cells isolated from the heart, as the only method that has provided satisfactory results is limited to co-culture with mature CMs. OT and some OT agonists, such as OT-Gly-Lys-Arg, that do not interfere with other physiological processes in the body (for example, without renal and hemodynamic effects), can successfully stimulate the differentiation of stem cell residing in the heart. In pathological conditions, such as cardiac ischemia and diabetes, this inducer can serve to stimulate the production of cardiac cells lost during the development of these pathologies. The advantage of such a therapy is supported by the fact that OT is produced endogenously in the organism, and does not have significant side effects when administered clinically. Moreover, it is now possible to inject (transplant) stem cells after previous stimulation with OT inducers, as in the case of heart attacks. Alternatively, direct treatment with OT molecules could promote cardiomyogenesis *in situ* and the regeneration of damaged hearts.

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12. References

Abdelalim, E. M., & Tooyama, I. (2009). BNP signaling is crucial for embryonic stem cell proliferation. *PLoS One, 4*(4), e5341.

Alizadeh, A. M., Faghihi, M., Sadeghipour, H. R., Mohammadghasemi, F., Imani, A., Houshmand, F., et al. (2010). Oxytocin protects rat heart against ischemia-reperfusion injury via pathway involving mitochondrial ATP-dependent potassium channel. *Peptides, 31*(7), 1341-1345.

Altstein, M., Whitnall, M. H., House, S., Key, S., & Gainer, H. (1988a). An immunochemical analysis of oxytocin and vasopressin prohormone processing in vivo. *Peptides, 9*(1), 87-105.

Altstein, M., & Gainer, H. (1988b). Differential biosynthesis and posttranslational processing of vasopressin and oxytocin in rat brain during embryonic and postnatal development. *J Neurosci, 8*(11), 3967-3977.

Antunes-Rodrigues, J., Favaretto, A. L., Gutkowska, J., & McCann, S. M. (1997). The neuroendocrine control of atrial natriuretic peptide release. *Mol Psychiatry, 2*(5), 359-367.

Baldini, P. M., De Vito, P., Vismara, D., Bagni, C., Zalfa, F., Minieri, M., et al. (2005). Atrial natriuretic peptide effects on intracellular pH changes and ROS production in HEPG2 cells: role of p38 MAPK and phospholipase D. *Cell Physiol Biochem, 15*(1-4), 77-88.

Bernatova, I., Rigatto, K. V., Key, M. P., & Morris, M. (2004). Stress-induced pressor and corticosterone responses in oxytocin-deficient mice. *Exp Physiol, 89*(5), 549-557.

Birkenfeld, A. L., Budziarek, P., Boschmann, M., Moro, C., Adams, F., Franke, G., et al. (2008). Atrial natriuretic peptide induces postprandial lipid oxidation in humans. *Diabetes, 57*(12), 3199-3204.

Bisping, E., Ikeda, S., Kong, S. W., Tarnavski, O., Bodyak, N., McMullen, J. R., et al. (2006). Gata4 is required for maintenance of postnatal cardiac function and protection from pressure overload-induced heart failure. *Proc Natl Acad Sci U S A, 103*(39), 14471-14476.

Bouchard, F., & Paquin, J. (2009). Skeletal and cardiac myogenesis accompany adipogenesis in P19 embryonal stem cells. *Stem Cells Dev, 18*(7), 1023-1032.

Breton, C., Di Scala-Guenot, D., & Zingg, H. H. (2001). Oxytocin receptor gene expression in rat mammary gland: structural characterization and regulation. *J Mol Endocrinol, 27*(2), 175-189.

Breton, C., Haenggeli, C., Barberis, C., Heitz, F., Bader, C. R., Bernheim, L., et al. (2002). Presence of functional oxytocin receptors in cultured human myoblasts. *J Clin Endocrinol Metab, 87*(3), 1415-1418.

Brownstein, M. J., Russell, J. T., & Gainer, H. (1980). Synthesis, transport, and release of posterior pituitary hormones. *Science, 207*(4429), 373-378.

Burbach, J. P., Luckman, S. M., Murphy, D., & Gainer, H. (2001). Gene regulation in the magnocellular hypothalamo-neurohypophysial system. *Physiol Rev, 81*(3), 1197-1267.

Calderone, A., Thaik, C. M., Takahashi, N., Chang, D. L., & Colucci, W. S. (1998). Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. *J Clin Invest, 101*(4), 812-818.

Cantley, L. C. (2002). The phosphoinositide 3-kinase pathway. *Science, 296*(5573), 1655-1657.

Cassoni, P., Marrocco, T., Bussolati, B., Allia, E., Munaron, L., Sapino, A., et al. (2006). Oxytocin induces proliferation and migration in immortalized human dermal microvascular endothelial cells and human breast tumor-derived endothelial cells. *Mol Cancer Res, 4*(6), 351-359.

Cattaneo, M. G., Chini, B., & Vicentini, L. M. (2008). Oxytocin stimulates migration and invasion in human endothelial cells. *Br J Pharmacol, 153*(4), 728-736.

Cattaneo, M. G., Lucci, G., & Vicentini, L. M. (2009). Oxytocin stimulates in vitro angiogenesis via a Pyk-2/Src-dependent mechanism. *Exp Cell Res, 315*(18), 3210-3219.

Christensen, G. (1993). Release of atrial natriuretic factor. *Scand J Clin Lab Invest, 53*(2), 91-100.

Dale, H. H. (1906). On some physiological actions of ergot. *Journal of Physiology, 34*, 163-206.

Danalache, B. A., Paquin, J., Donghao, W., Grygorczyk, R., Moore, J. C., Mummery, C. L., et al. (2007). Nitric oxide signaling in oxytocin-mediated cardiomyogenesis. *Stem Cells, 25*(3), 679-688.

De Vito, P., Di Nardo, P., Palmery, M., Peluso, I., Luly, P., & Baldini, P. M. (2003). Oxidant-induced pHi/Ca2+ changes in rat aortic smooth muscle cells. The role of atrial natriuretic peptide. *Mol Cell Biochem, 252*(1-2), 353-362.

Devost, D., Wrzal, P., & Zingg, H. H. (2008). Oxytocin receptor signalling. *Prog Brain Res, 170*, 167-176.

Dietz, J. R. (2005). Mechanisms of atrial natriuretic peptide secretion from the atrium. *Cardiovasc Res, 68*(1), 8-17.

Dimmeler, S., Zeiher, A. M., & Schneider, M. D. (2005). Unchain my heart: the scientific foundations of cardiac repair. *J Clin Invest, 115*(3), 572-583.

Dowell, J. D., Rubart, M., Pasumarthi, K. B., Soonpaa, M. H., & Field, L. J. (2003). Myocyte and myogenic stem cell transplantation in the heart. *Cardiovasc Res, 58*(2), 336-350.

Du Vigneaud, V. (1956). Hormones of the posterior pituitary gland. Oxytocin and vasopressin. *Harvey Lectures, 51*, 1-26.

Dudley, D. J. (1997). Oxytocin: use and abuse, science and art. *Clin Obstet Gynecol, 40*(3), 516-524.

Elabd, C., Basillais, A., Beaupied, H., Breuil, V., Wagner, N., Scheideler, M., et al. (2008). Oxytocin controls differentiation of human mesenchymal stem cells and reverses osteoporosis. *Stem Cells, 26*(9), 2399-2407.

Fathi, F., Murasawa, S., Hasegawa, S., Asahara, T., Kermani, A. J., & Mowla, S. J. (2009). Cardiac differentiation of P19CL6 cells by oxytocin. *Int J Cardiol, 134*(1), 75-81.

Favaretto, A. L., Ballejo, G. O., Albuquerque-Araujo, W. I., Gutkowska, J., Antunes-Rodrigues, J., & McCann, S. M. (1997). Oxytocin releases atrial natriuretic peptide from rat atria in vitro that exerts negative inotropic and chronotropic action. *Peptides, 18*(9), 1377-1381.

Florian, M., Jankowski, M., & Gutkowska, J. (2010). Oxytocin increases glucose uptake in neonatal rat cardiomyocytes. *Endocrinology, 151*(2), 482-491.

Fujio, Y., Nguyen, T., Wencker, D., Kitsis, R. N., & Walsh, K. (2000). Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. *Circulation, 101*(6), 660-667.

Furuya, K., Mizumoto, Y., Makimura, N., Mitsui, C., Murakami, M., Tokuoka, S., et al. (1995). A novel biological aspect of ovarian oxytocin: gene expression of oxytocin and oxytocin receptor in cumulus/luteal cells and the effect of oxytocin on embryogenesis in fertilized oocytes. *Adv Exp Med Biol, 395*, 523-528.

Gaddum, J. H. (1928). Some properties of the separated active principles of the pituitary (posterior lobe). *Journal of Physiology, 65*, 434-440.

Gassanov, N., Jankowski, M., Danalache, B., Wang, D., Grygorczyk, R., Hoppe, U. C., et al. (2007). Arginine vasopressin-mediated cardiac differentiation: insights into the role of its receptors and nitric oxide signaling. *J Biol Chem, 282*(15), 11255-11265.

Gassanov, N., Devost, D., Danalache, B., Noiseux, N., Jankowski, M., Zingg, H. H., et al. (2008a). Functional activity of the carboxyl-terminally extended oxytocin precursor

Peptide during cardiac differentiation of embryonic stem cells. *Stem Cells, 26*(1), 45-54.

Gassanov, N., Er, F., Zagidullin, N., Jankowski, M., Gutkowska, J., & Hoppe, U. C. (2008b). Retinoid acid-induced effects on atrial and pacemaker cell differentiation and expression of cardiac ion channels. *Differentiation, 76*(9), 971-980.

Gerstberger, R., Schutz, H., Luther-Dyroff, D., Keil, R., & Simon, E. (1992). Inhibition of vasopressin and aldosterone release by atrial natriuretic peptide in conscious rabbits. *Exp Physiol, 77*(4), 587-600.

Gimpl, G., & Fahrenholz, F. (2001). The oxytocin receptor system: structure, function, and regulation. *Physiol Rev, 81*(2), 629-683.

Gnecchi, M., & Melo, L. G. (2009). Bone marrow-derived mesenchymal stem cells: isolation, expansion, characterization, viral transduction, and production of conditioned medium. *Methods Mol Biol, 482*, 281-294.

Gutkowska, J., Jankowski, M., Lambert, C., Mukaddam-Daher, S., Zingg, H. H., & McCann, S. M. (1997). Oxytocin releases atrial natriuretic peptide by combining with oxytocin receptors in the heart. *Proc Natl Acad Sci U S A, 94*(21), 11704-11709.

Gutkowska, J., Jankowski, M., Mukaddam-Daher, S., & McCann, S. M. (2000). Oxytocin is a cardiovascular hormone. *Braz J Med Biol Res, 33*(6), 625-633.

Gutkowska, J., Miszkurka, M., Danalache, B., Gassanov, N., Wang, D., & Jankowski, M. (2007). Functional arginine vasopressin system in early heart maturation. *Am J Physiol Heart Circ Physiol, 293*(4), H2262-2270.

Gutkowska, J., & Jankowski, M. (2008). Oxytocin revisited: It is also a cardiovascular hormone. *J Am Soc Hypertens, 2*(5), 318-325.

Gutkowska, J., & Jankowski, M. (2009). Oxytocin: Old Hormone, New Drug. *Pharmaceuticals, 2*(3), 168-183.

Hassink, R. J., Brutel de la Riviere, A., Mummery, C. L., & Doevendans, P. A. (2003). Transplantation of cells for cardiac repair. *J Am Coll Cardiol, 41*(5), 711-717.

Hatami, L., Valojerdi, M. R., & Mowla, S. J. (2007). Effects of oxytocin on cardiomyocyte differentiation from mouse embryonic stem cells. *Int J Cardiol, 117*(1), 80-89.

Heineke, J., Auger-Messier, M., Xu, J., Oka, T., Sargent, M. A., York, A., et al. (2007). Cardiomyocyte GATA4 functions as a stress-responsive regulator of angiogenesis in the murine heart. *J Clin Invest, 117*(11), 3198-3210.

Hopkins, W. E., Chen, Z., Fukagawa, N. K., Hall, C., Knot, H. J., & LeWinter, M. M. (2004). Increased atrial and brain natriuretic peptides in adults with cyanotic congenital heart disease: enhanced understanding of the relationship between hypoxia and natriuretic peptide secretion. *Circulation, 109*(23), 2872-2877.

Houshmand, F., Faghihi, M., & Zahediasl, S. (2009). Biphasic protective effect of oxytocin on cardiac ischemia/reperfusion injury in anaesthetized rats. *Peptides, 30*(12), 2301-2308.

Husslein, P. (2002). Development and clinical experience with the new evidence-based tocolytic atosiban. *Acta Obstet Gynecol Scand, 81*(7), 633-641.

Iseri, S. O., Sener, G., Saglam, B., Gedik, N., Ercan, F., & Yegen, B. C. (2005a). Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. *Peptides, 26*(3), 483-491.

Iseri, S. O., Sener, G., Saglam, B., Gedik, N., Ercan, F., & Yegen, B. C. (2005b). Oxytocin protects against sepsis-induced multiple organ damage: role of neutrophils. *J Surg Res, 126*(1), 73-81.

Jain, M., Pfister, O., Hajjar, R. J., & Liao, R. (2005). Mesenchymal stem cells in the infarcted heart. *Coron Artery Dis, 16*(2), 93-97.

Jamali, M., Karamboulas, C., Rogerson, P. J., & Skerjanc, I. S. (2001). BMP signaling regulates Nkx2-5 activity during cardiomyogenesis. *FEBS Lett, 509*(1), 126-130.

Jankowski, M., Hajjar, F., Kawas, S. A., Mukaddam-Daher, S., Hoffman, G., McCann, S. M., et al. (1998). Rat heart: a site of oxytocin production and action. *Proc Natl Acad Sci U S A, 95*(24), 14558-14563.

Jankowski, M., Danalache, B., Wang, D., Bhat, P., Hajjar, F., Marcinkiewicz, M., et al. (2004). Oxytocin in cardiac ontogeny. *Proc Natl Acad Sci U S A, 101*(35), 13074-13079.

Jankowski, M. (2009). GATA4, a new regulator of cardiac fibroblasts, is sensitive to natriuretic peptides. *Cardiovasc Res, 84*(2), 176-177.

Jankowski, M., Bissonauth, V., Gao, L., Gangal, M., Wang, D., Danalache, B., et al. (2010a). Anti-inflammatory effect of oxytocin in rat myocardial infarction. *Basic Res Cardiol, 105*(2), 205-218.

Jankowski, M., Wang, D., Danalache, B., Gangal, M., & Gutkowska, J. (2010b). Cardiac oxytocin receptor blockade stimulates adverse cardiac remodeling in ovariectomized spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol, 299*(2), H265-274.

Kaneko, K., Fukuda, S., Ishikawa, S., & Saito, T. (1988). Comparative biological activities of alpha-rat and alpha-human atrial natriuretic peptide in the inhibition of arginine vasopressin release in conscious rats. *Endocrinol Jpn, 35*(6), 809-817.

Khairallah, R. J., Khairallah, M., Gelinas, R., Bouchard, B., Young, M. E., Allen, B. G., et al. (2008). Cyclic GMP signaling in cardiomyocytes modulates fatty acid trafficking and prevents triglyceride accumulation. *J Mol Cell Cardiol, 45*(2), 230-239.

Kiemer, A. K., & Vollmar, A. M. (2001). The atrial natriuretic peptide regulates the production of inflammatory mediators in macrophages. *Ann Rheum Dis, 60 Suppl 3*, iii68-70.

Kim, Y. S., Kwon, J. S., Hong, M. H., Kim, J., Song, C. H., Jeong, M. H., et al. (2010). Promigratory activity of oxytocin on umbilical cord blood-derived mesenchymal stem cells. *Artif Organs, 34*(6), 453-461.

Kobayashi, H., Yasuda, S., Bao, N., Iwasa, M., Kawamura, I., Yamada, Y., et al. (2009). Postinfarct treatment with oxytocin improves cardiac function and remodeling via activating cell-survival signals and angiogenesis. *J Cardiovasc Pharmacol, 54*(6), 510-519.

Laflamme, M. A., Zbinden, S., Epstein, S. E., & Murry, C. E. (2007). Cell-based therapy for myocardial ischemia and infarction: pathophysiological mechanisms. *Annu Rev Pathol, 2*, 307-339.

Lee, E. S., Uhm, K. O., Lee, Y. M., Kwon, J., Park, S. H., & Soo, K. H. (2008). Oxytocin stimulates glucose uptake in skeletal muscle cells through the calcium-CaMKK-AMPK pathway. *Regul Pept, 151*(1-3), 71-74.

Leor, J., Patterson, M., Quinones, M. J., Kedes, L. H., & Kloner, R. A. (1996). Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation, 94*(9 Suppl), II332-336.

Li, R. K., Mickle, D. A., Weisel, R. D., Mohabeer, M. K., Zhang, J., Rao, V., et al. (1997). Natural history of fetal rat cardiomyocytes transplanted into adult rat myocardial scar tissue. *Circulation, 96*(9 Suppl), II-179-186; discussion 186-177.

Light, K. C., Grewen, K. M., Amico, J. A., Brownley, K. A., West, S. G., Hinderliter, A. L., et al. (2005). Oxytocinergic activity is linked to lower blood pressure and vascular resistance during stress in postmenopausal women on estrogen replacement. *Horm Behav, 47*(5), 540-548.

Matsuura, K., Nagai, T., Nishigaki, N., Oyama, T., Nishi, J., Wada, H., et al. (2004). Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem, 279*(12), 11384-11391.

McBurney, M. W. (1993). P19 embryonal carcinoma cells. *Int J Dev Biol, 37*(1), 135-140.

McCann, S. M., Antunes-Rodrigues, J., Jankowski, M., & Gutkowska, J. (2002). Oxytocin, vasopressin and atrial natriuretic peptide control body fluid homeostasis by action on their receptors in brain, cardiovascular system and kidney. *Prog Brain Res, 139*, 309-328.

Menasche, P., Hagege, A. A., Scorsin, M., Pouzet, B., Desnos, M., Duboc, D., et al. (2001). Myoblast transplantation for heart failure. *Lancet, 357*(9252), 279-280.

Menasche, P., Hagege, A. A., Vilquin, J. T., Desnos, M., Abergel, E., Pouzet, B., et al. (2003). Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol, 41*(7), 1078-1083.

Menasche, P. (2009). Cell-based therapy for heart disease: a clinically oriented perspective. *Mol Ther, 17*(5), 758-766.

Michelini, L. C., Marcelo, M. C., Amico, J., & Morris, M. (2003). Oxytocinergic regulation of cardiovascular function: studies in oxytocin-deficient mice. *Am J Physiol Heart Circ Physiol, 284*(6), H2269-2276.

Miki, T., Miura, T., Tanno, M., Nishihara, M., Naitoh, K., Sato, T., et al. (2007). Impairment of cardioprotective PI3K-Akt signaling by post-infarct ventricular remodeling is compensated by an ERK-mediated pathway. *Basic Res Cardiol, 102*(2), 163-170.

Min, J. Y., Yang, Y., Converso, K. L., Liu, L., Huang, Q., Morgan, J. P., et al. (2002). Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol, 92*(1), 288-296.

Min, J. Y., Yang, Y., Sullivan, M. F., Ke, Q., Converso, K. L., Chen, Y., et al. (2003). Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. *J Thorac Cardiovasc Surg, 125*(2), 361-369.

Morris, M., Castro, M., & Rose, J. C. (1992). Alterations in oxytocin prohormone processing during early development in the fetal sheep. *Am J Physiol, 263*(3 Pt 2), R738-740.

Mouquet, F., Pfister, O., Jain, M., Oikonomopoulos, A., Ngoy, S., Summer, R., et al. (2005). Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. *Circ Res, 97*(11), 1090-1092.

Mukaddam-Daher, S., Yin, Y. L., Roy, J., Gutkowska, J., & Cardinal, R. (2001). Negative inotropic and chronotropic effects of oxytocin. *Hypertension, 38*(2), 292-296.

Mukaddam-Daher, S., Menaouar, A., Paquette, P. A., Jankowski, M., Gutkowska, J., Gillis, M. A., et al. (2009). Hemodynamic and cardiac effects of chronic eprosartan and moxonidine therapy in stroke-prone spontaneously hypertensive rats. *Hypertension, 53*(5), 775-781.

Murry, C. E., Wiseman, R. W., Schwartz, S. M., & Hauschka, S. D. (1996). Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest, 98*(11), 2512-2523.

Murry, C. E., Soonpaa, M. H., Reinecke, H., Nakajima, H., Nakajima, H. O., Rubart, M., et al. (2004). Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature, 428*(6983), 664-668.

Neuser, D., Stasch, J. P., Knorr, A., & Kazda, S. (1993). Inhibition by atrial natriuretic peptide of endothelin-1-stimulated proliferation of vascular smooth-muscle cells. *J Cardiovasc Pharmacol, 22 Suppl 8*, S257-261.

Nishimori, K., Takayanagi, Y., Yoshida, M., Kasahara, Y., Young, L. J., & Kawamata, M. (2008). New aspects of oxytocin receptor function revealed by knockout mice: sociosexual behaviour and control of energy balance. *Prog Brain Res, 170*, 79-90.

Noiseux, N., Gnecchi, M., Lopez-Ilasaca, M., Zhang, L., Solomon, S. D., Deb, A., et al. (2006). Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. *Mol Ther, 14*(6), 840-850.

Oliver, P. M., Fox, J. E., Kim, R., Rockman, H. A., Kim, H. S., Reddick, R. L., et al. (1997). Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci U S A, 94*(26), 14730-14735.

Ondrejcakova, M., Ravingerova, T., Bakos, J., Pancza, D., & Jezova, D. (2009). Oxytocin exerts protective effects on in vitro myocardial injury induced by ischemia and reperfusion. *Can J Physiol Pharmacol, 87*(2), 137-142.

Orlic, D., Hill, J. M., & Arai, A. E. (2002). Stem cells for myocardial regeneration. *Circ Res, 91*(12), 1092-1102.

Ott, I., & Scott, J. C. (1910). The action of infundibulin upon the mammary secretion. *Proceedings of the Society for Experimental Biology, 8*, 48-49.

Oyama, T., Nagai, T., Wada, H., Naito, A. T., Matsuura, K., Iwanaga, K., et al. (2007). Cardiac side population cells have a potential to migrate and differentiate into cardiomyocytes in vitro and in vivo. *J Cell Biol, 176*(3), 329-341.

Paquin, J., Danalache, B. A., Jankowski, M., McCann, S. M., & Gutkowska, J. (2002). Oxytocin induces differentiation of P19 embryonic stem cells to cardiomyocytes. *Proc Natl Acad Sci U S A, 99*(14), 9550-9555.

Paton, D. N., & Watson, A. (1912). The actions of pituitrin, adrenalin and barium on the circulation of the bird. *Journal of Physiology, 44*(5-6), 413-424.

Petersson, M. (2002a). Cardiovascular effects of oxytocin. *Prog Brain Res, 139*, 281-288.

Petersson, M., Lagumdzija, A., Stark, A., & Bucht, E. (2002b). Oxytocin stimulates proliferation of human osteoblast-like cells. *Peptides, 23*(6), 1121-1126.

Pfister, O., Mouquet, F., Jain, M., Summer, R., Helmes, M., Fine, A., et al. (2005). CD31- but Not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circ Res, 97*(1), 52-61.

Raeburn, C. D., Zimmerman, M. A., Arya, J., Banerjee, A., & Harken, A. H. (2002). Stem cells and myocardial repair. *J Am Coll Surg, 195*(5), 686-693.

Reversi, A., Cassoni, P., & Chini, B. (2005). Oxytocin receptor signaling in myoepithelial and cancer cells. *J Mammary Gland Biol Neoplasia, 10*(3), 221-229.

Rosenzweig, A. (2006). Cardiac cell therapy--mixed results from mixed cells. *N Engl J Med, 355*(12), 1274-1277.

Ruskoaho, H. (1992). Atrial natriuretic peptide: synthesis, release, and metabolism. *Pharmacol Rev, 44*(4), 479-602.

Russell, R. R., 3rd, Li, J., Coven, D. L., Pypaert, M., Zechner, C., Palmeri, M., et al. (2004). AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. *J Clin Invest, 114*(4), 495-503.

Sapino, A., Macri, L., Tonda, L., & Bussolati, G. (1993). Oxytocin enhances myoepithelial cell differentiation and proliferation in the mouse mammary gland. *Endocrinology, 133*(2), 838-842.

Shabbir, A., Zisa, D., Suzuki, G., & Lee, T. (2009). Heart failure therapy mediated by the trophic activities of bone marrow mesenchymal stem cells: a noninvasive therapeutic regimen. *Am J Physiol Heart Circ Physiol, 296*(6), H1888-1897.

Sirotkin, A. V., Florkovicova, I., Makarevich, A. V., Schaeffer, H. J., Kotwica, J., Marnet, P. G., et al. (2003). Oxytocin mediates some effects of insulin-like growth factor-I on porcine ovarian follicles. *J Reprod Dev, 49*(2), 141-149.

Skerjanc, I. S., Petropoulos, H., Ridgeway, A. G., & Wilton, S. (1998). Myocyte enhancer factor 2C and Nkx2-5 up-regulate each other's expression and initiate cardiomyogenesis in P19 cells. *J Biol Chem, 273*(52), 34904-34910.

Soares, T. J., Coimbra, T. M., Martins, A. R., Pereira, A. G., Carnio, E. C., Branco, L. G., et al. (1999). Atrial natriuretic peptide and oxytocin induce natriuresis by release of cGMP. *Proc Natl Acad Sci U S A, 96*(1), 278-283.

Stefanidis, K., Loutradis, D., Anastasiadou, V., Beretsos, P., Bletsa, R., Dinopoulou, V., et al. (2009). Embryoid bodies from mouse stem cells express oxytocin receptor, Oct-4 and DAZL. *Biosystems, 98*(2), 122-126.

Szeto, A., Nation, D. A., Mendez, A. J., Dominguez-Bendala, J., Brooks, L. G., Schneiderman, N., et al. (2008). Oxytocin attenuates NADPH-dependent superoxide activity and IL-6 secretion in macrophages and vascular cells. *Am J Physiol Endocrinol Metab, 295*(6), E1495-1501.

Takahashi, M., Li, T. S., Suzuki, R., Kobayashi, T., Ito, H., Ikeda, Y., et al. (2006). Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury. *Am J Physiol Heart Circ Physiol, 291*(2), H886-893.

Takayanagi, Y., Kasahara, Y., Onaka, T., Takahashi, N., Kawada, T., & Nishimori, K. (2008). Oxytocin receptor-deficient mice developed late-onset obesity. *Neuroreport, 19*(9), 951-955.

Taylor, D. A., Atkins, B. Z., Hungspreugs, P., Jones, T. R., Reedy, M. C., Hutcheson, K. A., et al. (1998). Regenerating functional myocardium: improved performance after skeletal myoblast transplantation. *Nat Med, 4*(8), 929-933.

Thibonnier, M., Conarty, D. M., Preston, J. A., Plesnicher, C. L., Dweik, R. A., & Erzurum, S. C. (1999). Human vascular endothelial cells express oxytocin receptors. *Endocrinology, 140*(3), 1301-1309.

Thompson, R. B., Emani, S. M., Davis, B. H., van den Bos, E. J., Morimoto, Y., Craig, D., et al. (2003). Comparison of intracardiac cell transplantation: autologous skeletal myoblasts versus bone marrow cells. *Circulation, 108 Suppl 1*, II264-271.

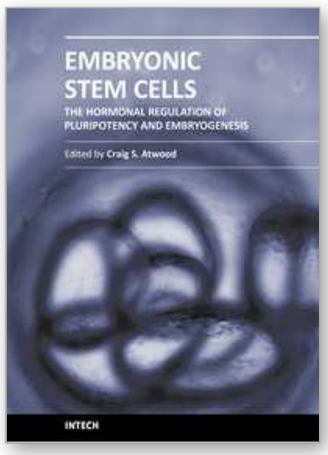
Toth, M., Vuorinen, K. H., Vuolteenaho, O., Hassinen, I. E., Uusimaa, P. A., Leppaluoto, J., et al. (1994). Hypoxia stimulates release of ANP and BNP from perfused rat ventricular myocardium. *Am J Physiol, 266*(4 Pt 2), H1572-1580.

Uchida, S., Fuke, S., & Tsukahara, T. (2007). Upregulations of Gata4 and oxytocin receptor are important in cardiomyocyte differentiation processes of P19CL6 cells. *J Cell Biochem, 100*(3), 629-641.

van der Heyden, M. A., & Defize, L. H. (2003). Twenty one years of P19 cells: what an embryonal carcinoma cell line taught us about cardiomyocyte differentiation. *Cardiovasc Res, 58*(2), 292-302.

Watanabe, E., Smith, D. M., Jr., Delcarpio, J. B., Sun, J., Smart, F. W., Van Meter, C. H., Jr., et al. (1998). Cardiomyocyte transplantation in a porcine myocardial infarction model. *Cell Transplant, 7*(3), 239-246.

Zingg, H. H., & Laporte, S. A. (2003). The oxytocin receptor. *Trends Endocrinol Metab, 14*(5), 222-227.



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