Biologic Proteins with Tocolytic Effects and Their Mechanisms: A Review

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ABSTRACT

Tocolysis is an important procedure in obstetrics used to delay preterm delivery. Tocolytics are medications used in achieving postponing preterm delivery mainly by reducing uterine contractility, hence, reducing perinatal morbidity and mortality. Different biologic proteins are involved. This essay discusses the effects of various biologic receptors and proteins on uterine relaxation, and their possible tocolytic mechanisms. These include the beta (β) adrenergic receptors, anoctamin-1, calcium channel antagonists, calmodulin, cyclo-oxygenase (COX) 1 and 2, amongst others. The articles used for this review covered the period up to 2022, and over 180 articles were obtained following literature search and of these, 64 were adapted for this article. Others whose scope were not relevant to the review were excluded. The articles were retrieved following searches using search engines and databases including Medline, Elsevier, Medscape, eMedicine, Google and PubMed. Understanding the mechanisms of tocolytic effects will benefit exploring more therapeutic ways of inducing uterine relaxation in improving pregnancy outcomes, thus resulting in decline of fetal morbidity and mortality.

Key Words: *Tocolytics, Preterm, Uterine relaxation, Receptors*

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INTRODUCTION

Tocolytic agents are substances or medications whose main goal is to stop or lessen myometrial smooth muscle cell spasms. Several pharmacological compounds, including oxytocin antagonists, β-adrenergic agonists, nonsteroidal anti-inflammatory medications, calcium channel antagonists, and magnesium sulfate, have had this inhibitory action examined in vitro, in vivo, or both [1-3]. However, the main objectives of tocolysis are to inhibit uterine contractions, prevent preterm delivery, prevent mortality associated with preterm birth and perinatal morbidity [4, 5].

Abnormal contractility in human may underlie such disorders as dysmenorrhea, endometriosis, improper embryo implantation, spontaneous miscarriage or preterm labor [6]. For contraction to take place, there are usually changes that are accompanied by decreasing of progesterone and increasing of estrogen, up regulation of myometrial oxytocin receptor, reduced nitric oxide activity increased the influx of calcium into myocyte and increased prostaglandins synthesis [7].

There are some benefits to extending pregnancy, which theoretically enables time for corticosteroids to be given to the mother to hasten fetal lung development and magnesium sulphate (MgSO4) to be given to lessen the risk of cerebral palsy [1, 3]. Because of these factors, brief tocolytic treatment is frequently used to prevent premature labor and delay preterm birth [8]. The ideal tocolytic agent is one that is safe for pregnant or non-pregnant women yet has no side effects on either she or her unborn child [9].

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Biologic proteins with tocolytic effects

Beta (β) adrenergic receptors (β-ARs)

The sympathetic nervous system is crucially dependent on beta-adrenergic receptors (β-ARs). They belong to the G protein-coupled receptor superfamily (GPCRs), and adrenaline and noradrenaline, two naturally occurring catecholamines, regulate their signaling pathway [10]. The smooth muscle of the uterus, bronchioles, and blood arteries is home to the β_2-AR. They prevent uterine contraction, vasodilation, and bronchodilation when stimulated [11]. Numerous human and animal tissues, including white and brown adipose tissues, skeletal muscles, the heart, gastrointestinal smooth muscles, the respiratory tract, and the urogenital system, have been found to contain β_3-adrenergic receptors (β_3-AR) [12]. The uterine β_3-AR perform the same function as β_2-AR; they are essential for the myometrium to relax, as demonstrated by studies on this subject [12, 13]. Functional β_3-AR has been detected in the myometrium of pregnant, near-term, and non-pregnant humans. They predominate over the β_2-AR subtype and are overexpressed in the uterus of pregnant women. Furthermore, β_3-AR are resistant to the long-term agonist-induced desensitisation of the myometrium at the conclusion of pregnancy in humans [14, 15].

The activation of Gs proteins by agonists of the β-adrenergic receptor is known to result in the relaxation of smooth muscle cells, and the Gs component of these proteins stimulates adenyl cyclase. In doing so, more cyclic adenosine monophosphate (cAMP) is produced. When protein kinase A is activated, the Ca^{2+} channels are then phosphorylated. This is a mechanism that happens in cardiomyocytes, and it might also happen in the myometrium of a pregnant woman. Adenylate cyclase is related to β-adrenergic receptors. β-adrenergic medications increase the intracellular concentration of cyclic AMP, which directly phosphorylates myosin light-chain kinase to inhibit its function. They also have the effect of lowering the calcium concentration inside cells. This led to mean actin removal and smooth muscle relaxation. Myosin-myosin interaction [16].

Anoctamin-1 (ANO1)

The ANO1 gene in humans [17] produces the protein known as anoctamin-1 (ANO1), also known as transmembrane member [16] A (TMEM16A). Anoctamin-1 functions as both a chloride channel and a bicarbonate channel, and it is a voltage-gated calcium-activated anion channel [18]. The apical iodide channel Anoctamin-1 is another feature. Throughout the gastrointestinal tract, this protein is found in smooth muscle, epithelial cells, olfactory sustentacular cells, vomeronasal neurons, and is highly abundant in interstitial cells of Cajal (ICC). All ANO family members (apart from ANO7) are expressed in the uterine smooth muscle (USM) tissue of pregnant women via messenger RNA (mRNA) [19]. When compared to non-pregnant USM, the expression of Anoctamin 1 mRNA was 15.2-fold lower in pregnant USM. Pregnant human USM tissue expresses anoctamin 1 protein. In a study on the pregnant human USM tissue using an organ bath, ANO1 antagonist benzbromarone was seen to attenuates the force and frequency of contractions that where oxytocin-induced [19, 20].

It is important to emphasise that because these channels are voltage-gated, their depolarizing threshold must be reached in order for them to become active. Uterine smooth muscle in mammals experiences a gestation-dependent hyperpolarization during the middle of pregnancy (and until the start of labor), which is known to be controlled by altered expression of potassium channels (USM). It is unknown what underlying ionic conductance causes the resting membrane potential in the USM to depolarize closer to the action potential (AP) threshold when a mammal reaches gestation and labour begins. One type of channel, the calcium-activated chloride channels (CaCCs), is thought to be crucial for the depolarizing drive (but not well characterised in the human uterus) [21]. In a study, it was demonstrated that blocking a subset of channels from the ANO family (also known as TMEM16), notably ANO1 and ANO2, reduced pro-contractile depolarizing membrane currents [20], resulting in a relaxation of murine USM contraction. Inhibition of contraction in the human USM during pregnancy results from ANO1 antagonistic effects such as pharmacological inhibition and genetic knockdown. A putative novel target for tocolysis is anoctamin 1 [19, 20].

Calcium channel antagonists

All currently available calcium antagonists share the common property of blocking the transmembrane flow of calcium ions through voltage-gated L-channels on non-vascular smooth muscles, vascular smooth muscles and non-contractile tissues. These nonvascular smooth muscles include uterine, bronchial, genitourinary, and gastrointestinal; vascular smooth muscles include arterial and venous; and non-contractile tissues include pituitary, pancreas, salivary glands, adrenal glands, gastric mucosa,

white cells, platelets, and lacrimal tissue [22]. Smooth muscle relaxes as a result of L-type channel blockade, and cardiac tissue experiences a detrimental inotropic effect [22]. Nifedipine and nicardipine are two of the most commonly used tocolytics [23, 24]. Calcium channel blockers (CCBs) are another class of drug. CCBs are steadily gaining ground and becoming more significant when compared to conventional tocolytics such beta-adrenergic receptor (-AR) blockers or magnesium sulfate [24].

The five different subunits of the Ca2+ channels (subunits 1, 2, 3, and 4) are complex proteins that are encoded by several genes [25]. The rise in Ca^{2+} concentration in the intracellular space of myometrial cells controls the contraction of the uterus. Voltage-gated Ca^{2+} channels (VGCCs) moderate Ca^{2+} inflow in response to membrane depolarization, which controls intracellular functions including contraction [26-30]. The myosin light chain kinase (MLCK) is activated in the myometrium cells as a result of the Ca^{2+} binding to calmodulin. As a result, serine 19 on myosin light chains is phosphorylated, starting the process that triggers cross-bridge cycling [25]. There are two origins of the rise in activator Ca^{2+}: release from the sarcoplasmic reticulum and/or entrance across the surface membrane via VGCCs. The primary source of Ca^{2+} for contraction in the uterus is due to the action potential's subsequent depolarization and opening of the VGCCs. When VGCCs are blocked, the influx of Ca^{2+} and contractions are halted [25]. Each contraction happens when a Ca^{2+} transient occurs in the uterus. Voltage-dependent L-type calcium channels have been discovered in uterine myometrium and are in charge of the bulk of the calcium current seen in human myometrium, according to electrophysiological, pharmacological, and molecular studies [25, 31].

Nifedipine is one example of a dihydropyridine (DHP) that binds to the binding side of the voltage-gated L-type channels of DHP, which is found on the α1 subunit. The α1 subunit's alternative splicing locations have produced a number of isoforms for the channels. One of the VGCC isoforms (S3B) has enhanced expression during labour in pregnant rats, according to research [25]. During pregnancy and labour in guinea pigs, significant alterations in the expression of the L-type VGCCs' α1 subunit were seen [25, 32]. The role of L-type VGCCs in the parturition process is aided by an increase in DHP binding capacity that was observed in the second half of gestation [25].

Calmodulin

The tiny heat- and acid-stable protein calmodulin was first identified in 1970. It was later found to be a binding protein that activates phosphodiesterase [33, 34]. Since then, it has been shown that the protein exists in the majority of organs, and its significance in the control of metabolism has been investigated. Calmodulin was found in the uterus of a bovine, and it is thought to have a significant role in smooth muscle contraction [35, 36]. It has also been demonstrated that calmodulin controls cell activity in other tissues: In cardiac cells, the Ca^{2+} dependent ATPase is involved in the intracellular transport of Ca^{2+}. Calmodulin influences this enzyme and enhances prostaglandin synthesis in human platelets, likely via activating phospholipase A2 [37].

In a study, calmodulin content in the uteri experienced a three-fold increase in both pregnant humans and rodents that had gotten to time of delivery compared to non-pregnant uteri [35, 38]. Another study found that there was no difference in the camodulin content between uteri with and without labour pain, indicating that even if camodulin levels rise at term, they do not directly trigger the start of delivery. The increase in camodulin content caused by estrogen has been demonstrated to augment the uterine contractile response [35, 39].

Cyclo-oxygenase (COX) 1 and 2

Cyclooxygenases (COXs) is a rate limiting enzyme whose major function is to regulate the process involved in the sythensis of prostaglandins (PGs), they are also key regulators of some of reproductive processes, such as ovulation, implantation, decidualization and parturition [40]. COX-1 and COX-2 are the two isoforms of this enzyme. By accelerating the transformation of arachidonic acid into prostaglandin G2, which is then further peroxidized to prostaglandins (PG2) [40, 41]. they regulate prostaglandins (PGs) sythensis. The majority of tissues express COX-1 as a constitutive enzyme, whereas COX-2 expression is activated by cytokines/growth factors, or inflammatory stimuli. According to studies, COX-1 is essential for parturition, whereas COX-2 is necessary for ovulation, fertilization, implantation, and decidualization [40, 42, 43].

Hormones called prostaglandins are recognised to have a wide range of uses. Prostaglandins influence uterine muscle contraction by elevating free intracellular calcium levels and activating myosin light chain kinase [44]. Prostaglandins are essential for beginning and sustaining labor [45]. The synthesis of prostaglandins depends on COX enzymes. Prostaglandin production will decrease, and uterine contractions will also diminish as a result of COX activity inhibition [40]. Indomethacin, the most commonly used prostaglandin inhibitor for tocolysis, works by reversibly binding to COX [9, 46]. The creation of COX-2-specific inhibitors was made possible by the recognition of COX's two different forms, COX-1 and COX-2. It is clear that COX-2 plays a role in triggering labour because COX-2 expression rises dramatically before labour begins but COX-1 expression is unchanged [46].

Prostaglandin inhibitors outperformed placebo in a network meta-analysis of tocolytic drugs in terms of their ability to delay parturition by 48 hours. They also had a 96% likelihood of being listed among the top three tocolytics in terms of effectiveness [9]. The ability of COX inhibitors to cross the placenta readily raises worries about the possible negative consequences of extended exposure on the developing fetus's gut, circulatory system, and kidney [46]. COX inhibitors have been shown to interfere with prostaglandin homeostasis. Numerous reports of oligohydramnios, renal failure, premature ductus arteriosus closure with subsequent pulmonary hypertension, persistent patent ductus arteriosus, necrotizing enterocolitis, and intraventricular haemorrhage in the fetus and neonate have been linked to in utero exposure to indomethacin during tocolysis [46, 47].

Prostaglandin receptors

Prostaglandins play an important role in female reproduction, they are inflammatory mediators. Prostaglandin (PG) receptors are expressed in the cytoplasmic membranes, they are heptahelical transmembrane G protein coupled receptors [48, 49]. Prostaglandins consist of PGF_{2a} PGD_2, PGE_2, thromboxane A_2 and prostacyclin [41]. While PG_{E2} acts through four distinct receptor subtypes, EP1, EP2, EP3, and EP4, PGF2a is mediated by FP receptors. The molecular characteristics, tissue location, and unique differential affinities to ligands of these receptors are all noteworthy. Female reproduction depends on the hormones PG_{E2} and PGF_{2a}, which have a variety of activities including uterine contraction, blastocyst spacing, implantation, and decidualization [49, 50]. EP1, EP3, and FP produce smooth muscle contraction, while EP2 and EP4 assist in smooth muscle relaxation [49, 51]. Endometrial blood flow, stromal edema control, and blood vessel permeability are expected to be affected by EP2, EP3, and EP4 [49].

In order to reproduce and keep a pregnancy going, prostaglandins, which are lipid mediators, are essential. The terminal prostanoid synthase, PGE synthase (PGES), is capable of enzymatically converting PGH_2—the end product of cyclooxygenase—to PGE_2. The functionally different cell surface receptors EP1, EP2, EP3, and EP4 are engaged by PG_{E2} and bind to and become active. PGF_{2a} is regarded as the most likely candidate among the prostaglandins to be present throughout pregnancy. By enhancing oxytocin-induced contractions, it is essential for the myometrium to function properly during parturition. PGF synthase produces PGF_{2a}, which has an effect through the FP receptor [40].

ATP-sensitive K^+ channels (K_{ATP} channels) and Receptors

Potassium channels plays a vital roles in normal reproductive function, this function depends primarily on it, and the ATP-sensitive K^+ (K_{ATP}) channels plays a leading role in it, as seen lately in a number of studies [52-55]. When potassium channels are activated they increase the plasma membrane potential, this blocks the voltage-dependent Ca^{2+} channels and prevents Ca^{2+} entry, thus stopping contraction and cause relaxation of myometrium muscle fibers [56]. Even if they are expressed constitutively in the myometrium, they are down regulated in late pregnancy, to ensure higher excitability of the uterus during labor [53, 57]. These facts shows the substantial role the K_{ATP} channels plays in the regulation of excitation and contraction coupling in myometrium. Estrogen and progesterone regulates K_{ATP} channels. K_{ATP} channels in the uterus and other smooth muscles are activated by estrogen, however, an opposite effect is seen in pancreatic cells and cardiomyocytes [58-61] and the most reason for this is that estrogen in physiological concentrations moderately increases production of nitric oxide (NO) [62].

Bitter taste receptors (TAS2Rs)

The bitter taste receptors (TAS2Rs) and its canonical signaling elements (i.e., G-protein gustducin and phospholipase C β2) are highly expressed in the myometrial cells of both humans and mice [63]. According to studies, bitter tastes have the power to relax myometrium that has been pre-contracted by various uterotonics. More frequently than other frequently used tocolytics, chloroquine (a phenotypical bitter tastant) was able to stop contractions in preterm deliveries in mice that were caused by the progesterone receptor antagonist mifepristone or the bacterial endotoxin LPS [63]. Relaxation of myometrium, initially contracted by various contractile agonists, was activated by the canonical TAS2R signaling system, hence, one possible area to explore in developing effective tocolytics for preterm birth management will be targeting the TAS2Rs [63].

Heat shock proteins (HSPs)

In the early 1960s, Ferruccio Ritossa first described the heat shock proteins (HSP) while working with *Drosophila melanogaster* [64]. A wide family of molecular chaperones known as the heat shock proteins (HSPs) are categorised based on their molecular weights (HSP27, HSP40, HSP60, HSP70, and HSP90). They carry out several physiological and defensive functions that aid in the preservation of cellular homeostasis [65, 66]. They are swiftly upregulated in response to exposure to stressful conditions [66].

There is an abundance of HSP27, HSP60, HSP70 and HSP90 in endometrial and uterine cells, an indication of their likely involvement during the pregnancy process [64, 67]. They are linked with decidualization, implantation and placentation, with their dysregulation associated with

pathological pregnancies [64]. When circulating Hsp70 concentrations increases there is a corresponding increase in the risk of several pregnancy complications [67]. Elevated circulating heat shock proteins are associated with spontaneous preterm birth, thus, their suppression maybe positive in positive pregnancy outcomes [68, 69].

CONCLUSION

Tocolysis involves the use of medications to cause uterine relaxation with the purpose of delaying fetal delivery following preterm contractions. Understanding the mechanisms of tocolytic effects will benefit exploring more therapeutic ways of inducing uterine relaxation in improving pregnancy outcomes, thus resulting in decline of fetal morbidity and mortality.

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