Zagazig University Medical Journal
www.zumj.journals.ekb.eg

ORIGINAL ARTICLE

Apelin Induced Modulation of Uterine Contractility in Adult Albino Rats and its Possible Mechanism/s of Action

Ali Khalil Asalah, Shreif Wagih Mansour, Kamelia Ibrahim Attia, Somya El sayed Mohammed

Physiology Department, Faculty of Medicine, Zagazig University,Zagazig, Egypt

Corresponding author
Somya El sayed Mohammed
Physiology Department, Faculty of Medicine, Zagazig University,Zagazig, Egypt

somyamahdy @ gmail.com

Submit Date 2019-03-29
Revise Date 2019-04-09
Accept Date 2019-04-09

ABSTRACT

Background: Apelin is an endogenous ligand for the G protein-coupled receptor APJ. The expression of both apelin and APJ has been detected in a variety of tissues including heart, ,ovary , placenta and uterus. It has a relaxant effect on smooth muscles of the stomach and blood vessels. However , its effect on the smooth muscle of the uterus is still controversy and its mechanism of action is not fully investigated. This study was designed to demonstrate the in vitro effects and mechanism of action of apelin on rat uterine reactivity. **Methods:** 60 adult albino rats (48 females and 12 males). Female rats were randomly divided into non- pregnant, pregnant (day 6 and day 19) and 1st day postpartum groups. The effects of apelin (1, 10 and 100 nmol /L) on spontaneous reactivity of isolated uterine strips were studied. Also, apelin (100 nmol/L) in the presence of L-NAME, apamin and Glibenclamide was investigated. **Results:** Apelin exerted a significant dose dependent reduction in frequency and amplitude of spontaneous uterine contraction. This reduction was significantly more potent on day 6 than that in both non pregnant and pregnant rats on day 19. Also , it was significantly and nearly completely abolished in the presence of L-NAME, but it was significantly and partially decreased in the presence of (Apamin) and (Glibenclamide). **Conclusions**: Apelin has a potent utero-relaxant effect which is greater in early pregnancy compared with late pregnancy. Thus Apelin may be a promising tocolytic drug.

Keyword: Apelin ; uterine contractility; pregnancy.

INTRODUCTION

Apelin is a an adipokine and the endogenous ligand for APJ, an angiotensin-1-like receptor. It has been isolated from the bovine stomach**,** before the isolation of apelin, the APJ receptor was referred to as an orphaned G-protein-coupled receptor (GPCR) because its endogenous ligand was unidentified [1].

Apelin and APJ were detected in various tissues and organs such as stomach, brain, heart, lung, uterus, ovary and produced in pregnant and lactating breast, high levels were identified in the placenta suggesting a possible placental origin of apelin in pregnancy [2].

Apelin acts via APJ to mediate effects on the cardiovascular system, fluid homeostasis, glucose metabolism and food intake influencing not only cyclic AMP production but also protein kinase c (PKC) , phosphatidyl inositol 3 kinase (PI3K), protein kinase B (Akt), extracellular regulated kinase (ERK) and cytoplasmic Ca^{2+} concentration**.** Apelin exerts an inotropic effect on the heart and simultaneously elicits vasodilatation in the peripheral circulation [3]. The myometrial smooth muscle, as a nonvascular smooth muscle, appears to respond to apelin exposure in a similar way as vascular smooth muscle does [4].

There are contradictory reports about the effect of apelin on uterine contractility Hehir & Morrison [5] observed that apelin exerts an inhibitory effect on human myometrial contractility in vitro, in tissue obtained during pregnancy. In contrast, Kacar et al. [6] reported that apelin induces myometrial contractions.

METHODS

Material:

60 adult albino rats (48 female rats and 12 male rats) with an average weight, 180-200 grams . The male rats were used for induction of pregnancy. Female groups randomly divided in to (1): non-pregnant, (2a): Early pregnant at day 6 of gestation. (2b):Late pregnant at day 19 of gestation and (3) : 1st day post-partum .

The experimental protocol was approved by physiology department and by local medical ethics committee in faculty of medicine of Zagazig University (Institutional Review Board, IRB , ZU-IRB 1757-21-12-2014).

All animal experiments were with the ARRIVE guidelines and carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments.

Drugs and chemicals:

Apelin-13 trifluoroacetate salt, L-NAME, N^G-nitro-L-arginine methyl ester nitric oxide synthase inhibitor , Apamin, small conductance Ca^{2+} activated K^+ channels blocker and Glibenclamide , ATP sensitive K^+ channel blocker (Sigma- Aldrich co. USA).

De Jalone solution gm/2 L (NaCl, 18; KCl, 0.84; Glucose,1;Na HCO_3,1; $CaCl_2$,0.4,El Nasr Pharmaceutical Chemicals CO. Abu Zaabal, Egypt.) pH7.4 bubbled with Carbogen (95%O2 and 5% CO2) to be used as a bath fluid for isolated uterine strips.

Methods:

Preparation of the non -pregnant group

The non-pregnant female rats were prepared with subcutaneous injection of diethylstilbestrol (0.5 mg/ kg) 24h before the experiment started for sensitization of the uterine smooth muscle **[7].**

Timed- pregnant group:

Determination of the first day of pregnancy:

Vaginal smears were examined daily by using light microscope to ensure regular estrus cycle. The estrus phase was detected by the presence of cornified epithelial cells [8].

The female proved to be in estrus phase was paired with a mature male rat in a separate cage. After mating, females were subsequently isolated until the time of analysis to ensure accurate gestation timing, and in the next morning a vaginal smear taken. Copulation was confirmed by the presence of a copulation plug or spermatozoa in the vagina. The presence of sperms indicated the first day of gestation [9]. Parturition usually occurs in the evening of day 21 or the morning of day 22 as the duration of pregnancy in rats is about 21 days **[10].**

3- Isolated uterine tissue protocol:

Female rats were sacrificed under light ether anesthesia by decapitation. The abdomen was opened, the uterine horns were dissected, and transferred immediately to a dish containing De-Jalone solution, then the extraneous tissues were removed e.g. pregnant uteri were cleaned from fat, placenta, fetus, fetal membrane and then rinsed thoroughly. Afterwards each horn was opened longitudinally along its mesenteric border and divided by a long cut into two equal length segments to produce strips of about 0.4 cm in width x 1.3 cm in length [11]. The strips were mounted in De Jalone solution (25 ml volume).

One end of the strip was attached to a fixed pin in the aerator of the bath , the other end was fixed through a thread to an isotonic transducer (AD Instruments , Spain) connected to Bridge Amp amplifiers (AD Instruments, Australia), with 4 channel data acquisition system (Power Lab/4/30, AD Instruments) connected to computer, data were saved using the program Chart 7.2

After recording the effect of each dose, the uterine strips were washed 2 to 3 times with 5 minutes interval and left for about half an hour to return to their inherited conditions.

The drugs were added as follow: *apelin* (1, 10 and 100 nmol/L) [5] to organ baths containing uterine strips . In additional experiments, the contractile activity of the uterine strips isolated from rats on day 6 of gestation was recorded in response to addition of the third dose of *apelin* (100 nmol/L) in the presence of L-NAME ,(3×10^{-5} mol/L)[12] , Apamin (10^{-8} mol/L) [13] and Glibenclamide (10^{-6} mol/L) [14].

Strips were incubated for 15 min with each of the previously mentioned chemicals

followed by a period of 2-5 min incubation with apelin (100 nmol/ L).

The amplitude (mm) and frequency (cycle/20min) of contractions developed were quantitated and expressed as the percentage of reduction.

Statistical analysis:

Data were presented as mean ± SD. Statistical significance was determined by paired "t" test for differences within the same group. Differences between groups were determined by a one-way ANOVA and correlation coefficient (r). *P*<0.05 was considered statistically significant. SPSS version (14) program for Windows (SPSS Inc. Chicago, IL, USA) was used.

RESULTS

Tables(1a,b) and Figure (1) demonstrate the effect of different doses of apelin (1, 10 and 100 nmol/L) on spontaneous contractility of uterine strips isolated from non-pregnant, pregnant rats on day 6, on day 19 of gestation and 1st day post-partum.

It was found that apelin had a significant utero-relaxant effect as it produced a significant decrease in the amplitude and frequency of spontaneous uterine contraction.

This relaxant effect was found to be dose dependant because there was a significant positive correlation between the relaxant effect and the doses used.

Table (2) and Figures (2a,b) show a comparison between the percentages of reduction ($\overline{X}$ ±SD) of amplitude and frequency of spontaneous contraction the presence of different doses of apelin (1 , 10 and 100 nmol/L) .

It was observed that the utero-relaxant effect of apelin was significantly higher pregnant rats on day 6 of gestation when compared with non -pregnant , pregnant rats on day 19 and 1st day post -partum . However, a non-significant difference was observed when comparing the non-pregnant with the pregnant on day 19 and 1st day post -partum.

Figure (3 ,4) show the effect of apelin (100 nmol/L) on spontaneous contractility of uterine strips isolated from pregnant rats on day 6 of gestation in the presence of nitric oxide synthase inhibitor L-NAME, ($3x10^{-5}$mol/L), Apamin, (10^{-8} mol/L) and Glibenclamide, (10^{-6} mol/L).

Table 1a. Effect of different doses of apelin (1, 10, 100 nmol/L organ bath fluid) on the amplitude (mm) of spontaneous contraction of uterine strips isolated from all studied group.

Amplitude (mm)									
	Group 1(non-pregnant)								
	1 nmol/L organ bath fluid			**10 nmol/L organ bath fluid**			**100nmol/L organ bath fluid**		
	Before	**After**	**% of change**	**Before**	**After**	**% of change**	**before**	**After**	**% of change**
$\overline{X}$	6.25	5.75	10	7.08	3.83	45.97	6.50	1.08	68.84
±SD	1.38	1.21	10.44	1.44	0.93	6.93	1.24	0.79	12.09
"t" **P**	3.31 NS (p > 0.05)			14.93 (p < 0.05			17.31 (p < 0.05)		
r	0.948 (p < 0.001)								
Group 2a (Early pregnant at day 6 of gestation)									
$\overline{X}$	16.50	13.16	20.43	16.83	6.75	60	15.58	2.08	88.19
±SD	1.78	1.94	5.15	1.1	1.6	8.28	1.78	1.67	9.33
"t" **P**	14.83 (p < 0.05)			24.2 (p < 0.01)			40.04 (p < 0.001)		
r	0.961(p < 0.001)								
Group 2b (Late pregnant at 19 day of gestation)									
$\overline{X}$	17.75	15.91	10.2	18.4	11.00	40	17.25	4.6	72.84
±SD	2.00	1.97	4.15	2.5	1.8	6.09	1.95	1.07	6.2

"t" **P**	8.84 NS (p > 0.05)			16.4214.83 (p < 0.05)			23.79(p < 0.001)		
r	0.979(p < 0.001)								
Group 3(1st day post-partum)									
$\overline{X}$	11.16	10.16	9.4	9.58	5.6	40.4	9	1.6	70.16
±SD	2.03	2.28	8.68	1.37	0.77	7.27	1.2	1.3	10.07
"t" **P**	4.06 NS (p > 0.05)			11.65 (p < 0.05)			13.32 (p < 0.001)		
r	0.943(p < 0.001)								

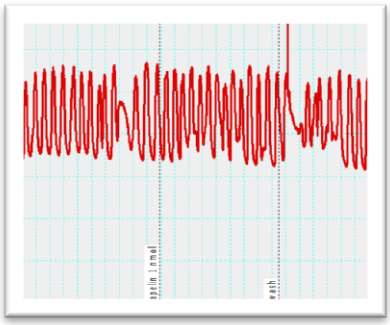
Table 1b. Effect of different doses of apelin (1, 10, 100 nmol/L organ bath fluid) on the frequency **(cycle /20 min)** of spontaneous contraction of uterine strips isolated from all studied groups.

Frequency (cycle /20 min)									
	Group 1(non-pregnant)								
	1 nmol/L organ bath fluid			**10 nmol/L organ bath fluid**			**100nmol/L organ bath fluid**		
	Before	**After**	**% of change**	**Before**	**After**	**% of change**	**Before**	**After**	**% of change**
$\overline{X}$	16	15.25	4.92	17	8.50	48.94	16.66	3.75	78.33
SD	1.85	2.22	4.91	1.47	1.16	5.98	1.49	2.59	14.56
"t" **P**	3.44 (p < 0.05)			21.31 (p < 0.05)			26.69 (p < 0.05)		
R	0.951(p < 0.001)								
Group 2a (Early pregnant at day 6 of gestation)									
$\overline{X}$	18.08	14.58	19.83	15.83	4.9	69.1	17.16	1.58	91.47
±SD	1.37	1.62	5.15	2.36	1.16	4.3	2.32	1.24	6.34
"t" **P**	13.40 (p < 0.05)			25.12 (p < 0.001)			39.14 (p < 0.001)		
R	0.936(p < 0.001)								
Group 2b (Late pregnant at 19 day of gestation)									
$\overline{X}$	18.4	17.5	5.1	18	12	50	18.75	4.33	72.84
±SD	1.44	1.67	4.5	2.00	1.8	4.76	2.09	0.77	6.23
"t" **P**	4 NS (p > 0.05)			28.14 (p < 0.05)			28.14 (p < 0.001)		
R	0.983(p < 0.001)								
Group 3 (1st day post-partum)									
$\overline{X}$	18.3	17.6	3.46	18.25	11.66	46.18	18.25	4	78.35
±SD	1.43	1.15	4	1.4	1.6	5.68	1.4	2	10.65
"t" **P**	2.96NS (p > 0.05)			22.89 (p < 0.05)			27.97 (p < 0.001)		
R	0.972(p < 0.001)								

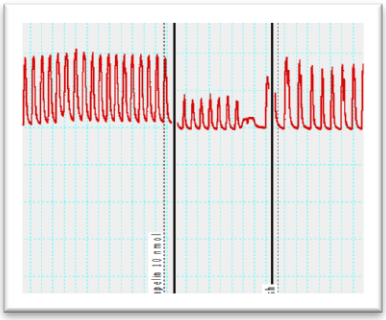
NB: r = correlation between dose of apelin **(**nmol/L organ bath fluid) and percentage of reduction ($\overline{X} \pm$ SD) of amplitude and frequency of contraction of uterine strips.
NS = Non Significant

Table 2. The percentage of reduction ($\bar{X}$ **±SD)** of amplitude and frequency of spontaneous contraction of uterine strips isolated from non-pregnant , rats on day 6 , day 19 of pregnancy and 1st day post -partum under the effect of different doses of apelin.

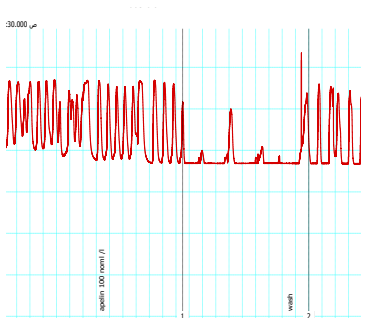
N = 12	*Percentage of reduction in the amplitude (mm)*											
	1 nmol/ L				10 nmol /L				100 nmol /L			
	non pregnant	Day 6 of gestation	Day 19 of gestation	1st day postpartum	non pregnant	Day 6 of gestation	Day 19 of gestation	1st day postpartum	non pregnant	Day 6 of gestation	Day 19 of gestation	1st day postpartum
$\bar{X}$	10	20.43	10.2	9.4	38.97	60	42	40.4	68.84	88.19	72.84	70.16
±SD	10.44	5.15	4.15	8.68	6.93	8.28	6.09	7.27	12.09	9.33	6.2	10.07
F	5.85 (p< 0.001)				20.6 (p < 0.001)				8.31 (p< 0.001)			
P of LSD VS non-preg		< 0.001	NS	NS		< 0.001	NS	NS		< 0.05	NS	NS
P of LSD VS early preg.			< 0.001	< 0.001			< 0.001	NS			< 0.01	< 0.02
P of LSD VS late preg.				NS				NS				NS
Percentage of reduction in frequency (cycle /20 min)												
$\bar{X}$	4.92	19.83	5.1	3.46	48.94	69.1	50	46.18	70.32	91.47	75.43	68.35
±SD	4.91	5.15	4.5	4	5.98	4.3	4.76	5.68	10.56	6.34	6.23	8.74
F	30.67 (p < 0.001)				113.28 (p < 0.001)				5.7(p < 0.05)			
P of LSD VS non-preg.		< 0.001	NS	NS		< 0.001	NS	NS		< 0.05	NS	NS
P of LSD VS early preg.			< 0.001	< 0.001			< 0.001	NS			< 0.01	< 0.02
P of LSD VS late preg.				NS				NS				NS



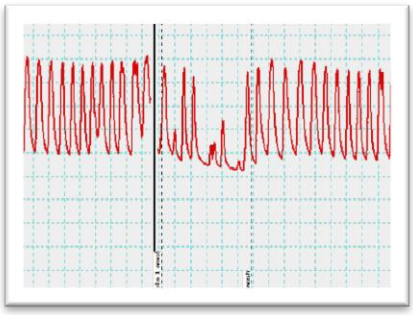
(A)



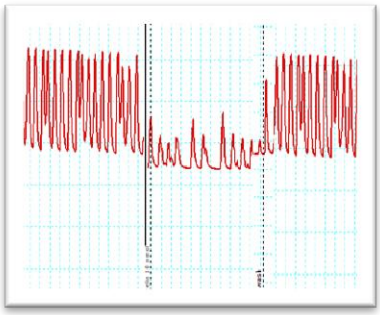
(B)



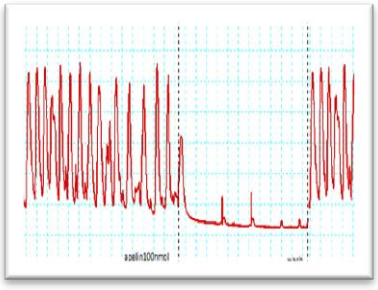
(C)



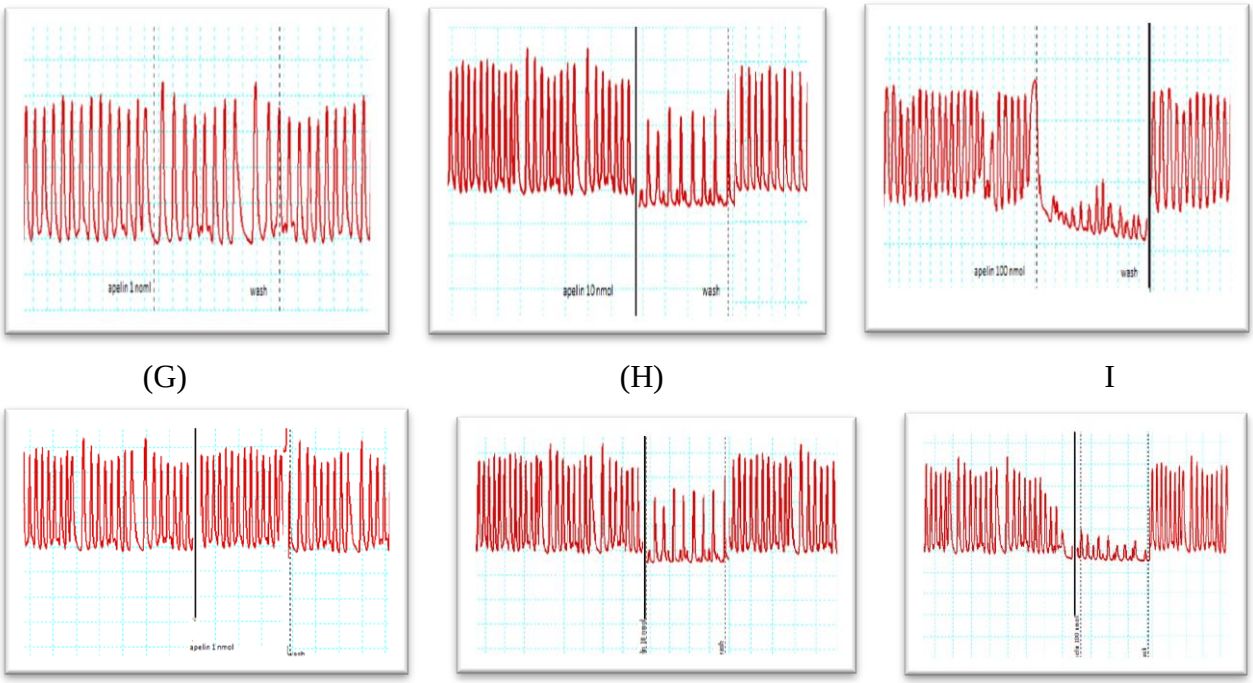
(D)



(E)



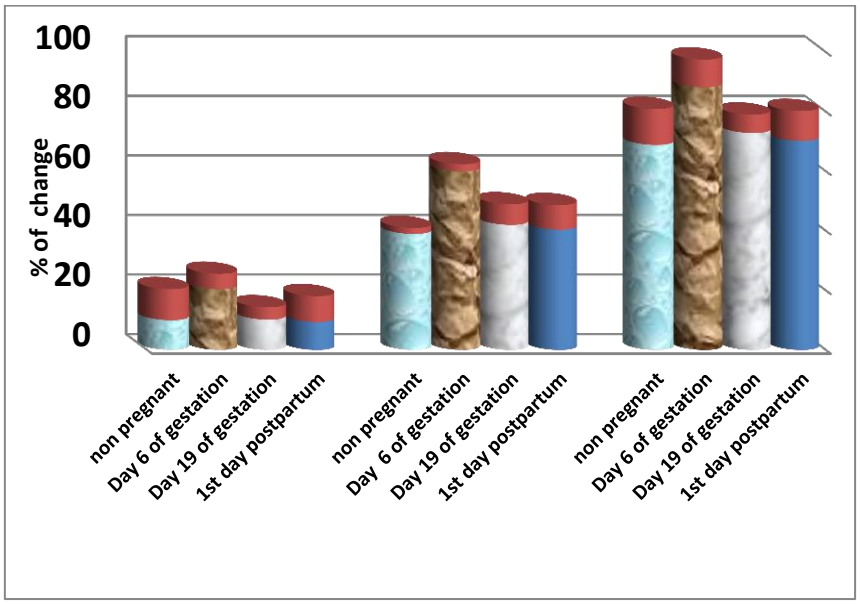
(F)



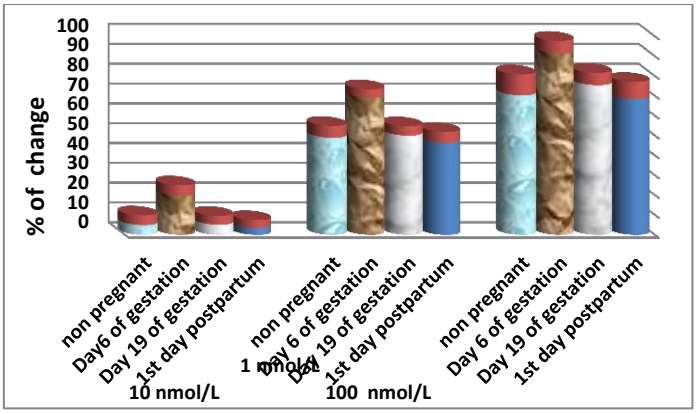
(G) (H) I

(J) (K) (L)

Figure 1. Representative recordings demonstrating the effect of different doses of apelin (1, 10, 100 nmol/L) on spontaneous contractility of uterine strips isolated from non-pregnant (A,B,C)pregnant rats on day 6 (D,E ,F) , pregnant rats on day 19 of gestation (G ,H ,I) and 1st day post-partum (J, K , L).



(a)



(b)

Fig 2a,b. The percentages of reduction ($\overline{X}$ **±SD)** of amplitude (a) and frequency (b) of spontaneous contraction of uterine strips isolated from non -pregnant rats, rats on day 6 , day 19 of gestation and 1st day post-partum in the presence of different doses of apelin (1st dose 1, 2nd dose 10 and 3rd dose 100 nmol/L organ bath fluid).

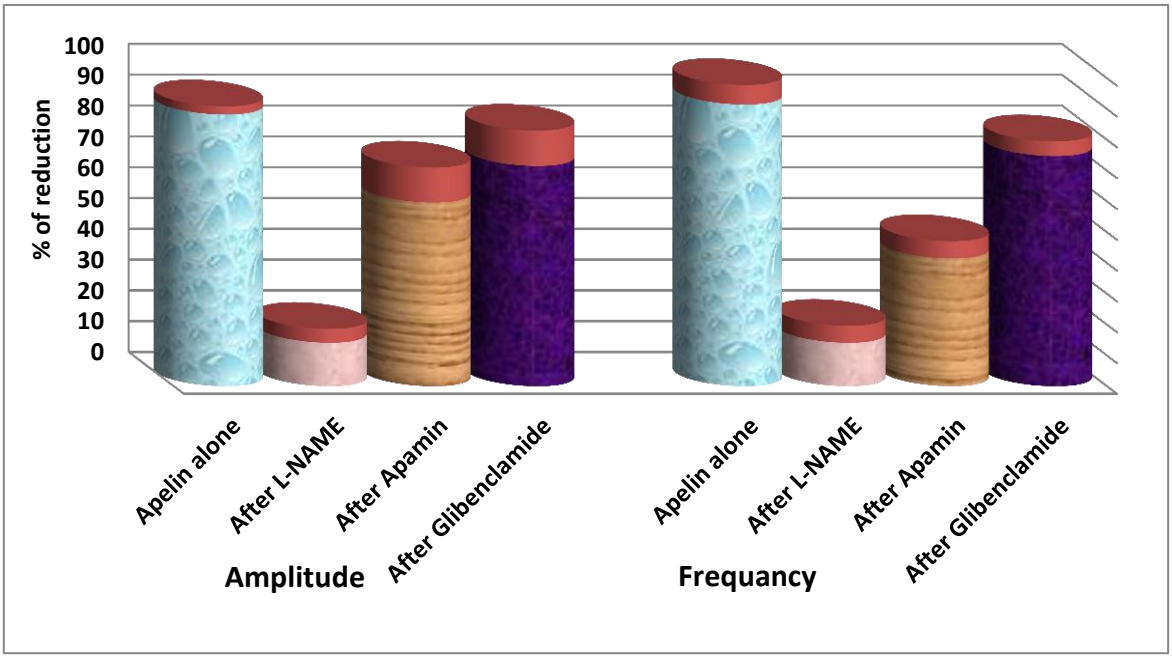
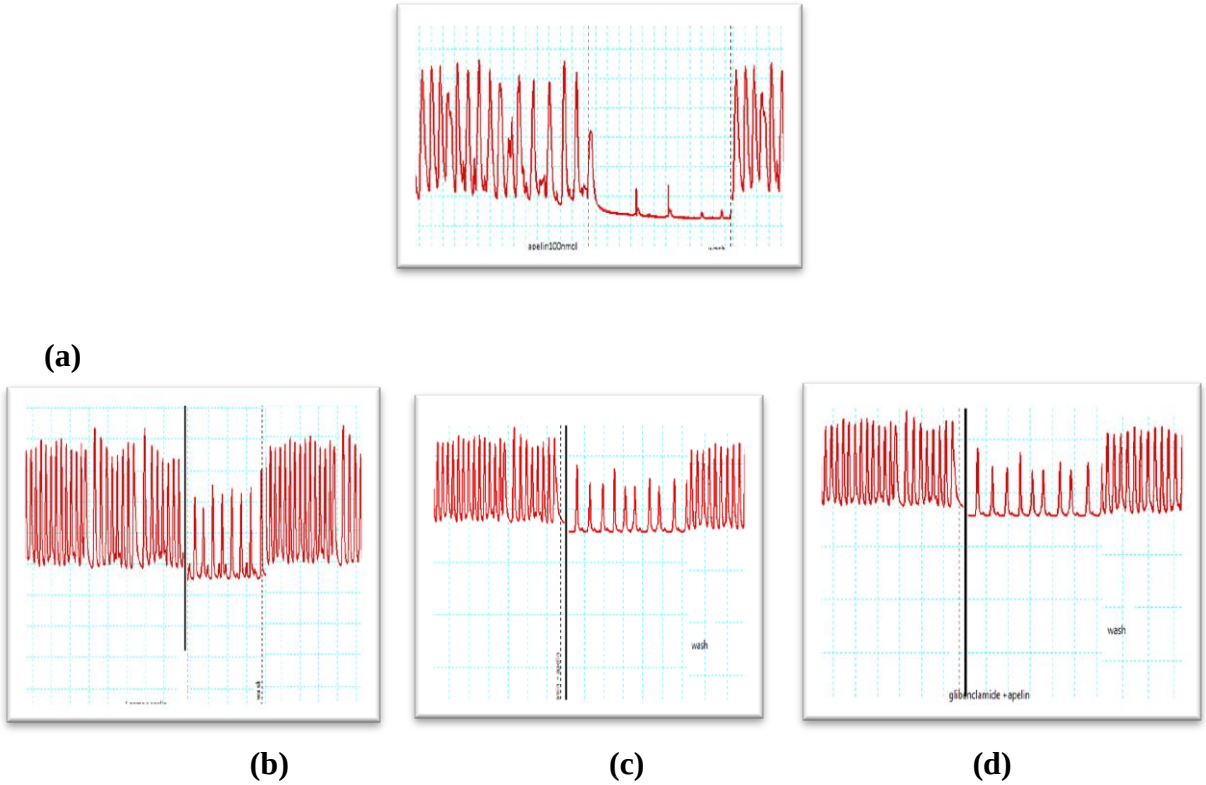


Figure (3): The percentages of reduction ($\overline{X}$ **±SD)** of amplitude (a) and frequency (b) of contraction produced by addition of apelin (100 nmol/L organ bath fluid) to uterine strips isolated from pregnant rats (day 6) before and after incubation with L-NAME (3×10^{-5} mol/L), Apamin (10^{-8} mol/L) and Glibenclamide, (10^{-6} mol/L**)**



(a) **(b)** **(c)** **(d)**

Figure (4): Representative recordings demonstrating the effect of apelin (100 nmol/L organ bath fluid) **(a)** on spontaneous contractility of uterine strips isolated from pregnant rats on(day 6)of gestation in the presence of, L-NAME, ($3x10^{-5}$ mol/L) **(b),** Apamin, (10^{-8} mol/L) **(c)** and Glibenclamide, (10^{-6} mol/L) **(d).**

DISCUSSION

The results of the present study demonstrated that apelin addition to organ bath fluid was associated with a significant decrease in both frequency and amplitude of spontaneous contractions of uterine strips isolated from pregnant, non-pregnant and 1st day post-partum rats. This effect was dose dependent and was observed at relatively low concentrations for in vitro experiments (in nanomolar range).

Our results are in agreement with the results of Hehir and Morrison [5] who studied the effect of apelin on human uterine strips isolated from pregnant women in the 3rd trimester during performance of elective cesarean section and found that addition of apelin produces a significant reduction in frequency and amplitude of both spontaneous and oxytocin induced isometric contraction .

However, at variance with those of Kacar et al. [6] who observed that addition of apelin to organ bath fluid containing strips isolated from pregnant rat uterus at day 21 produced a significant increase in both frequency and amplitude of uterine contraction and showed that this effect occurred in Ca^{2+} free medium and did not occur in presence of protein kinase C inhibitor suggesting that PKC pathway but not extracellular Ca^{2+} might play a role in these mechanism. They postulated that apelin might be an endogenous peptide that play a role on uterine contraction at birth in rats facilitating parturition.

The effect of apelin may be attributed to apelin induced release of NO which may be mediated by increased activity of NOS after binding of apelin to APJ . This was proved by the observation that the apelin utero relaxant effect was nearly abolished when apelin was added after incubation of uterine strips isolated from pregnant rats on day 6 of gestation with L- NAME.

The pregnant uterus is relatively relaxed and quiet while enlarging to accommodate the developing fetus. NO has a relaxant effect on myometrium, NO maintains relative uterine quiescence throughout gestation**.** NO is synthesized in a variety of tissues, including rat uterus, from L-arginine by NO synthase (NOS) [15].

The apelin effect was greater in early than in late pregnancy and this may be attributed to the increased uterine NOS activity and /or expression which may be hormonal dependent. Also, explained by up regulation of APJ by hypoxia inducible factor -1α and pro inflammatory factor [16]. Progesterone regulates NOS and NO production during gestation. Not only progesterone affect the generation of NO, but it may also up-regulate its effects [17]. In addition, Figuero and massmann [18] reported that estrogen increased the NOS activity in the uteri of sheep and rats. Also, Magness et al. [19] showed that Increased estradiol was associated with vaso-relaxation and increased expression and activity of vascular NOS**,** they also observed a biphasic effect of estrogens on constitutive NOS activity with stimulatory effects in early pregnancy and inhibitory effects in late pregnancy**.**

It was suggested that the effects of estrogens are mediated through a calcium-dependent mechanism. Many studies addressed the dose-related effects of estradiol on uterine NO production. Thus, it appears that low doses of estradiol stimulate NO production and high doses either inhibit or had no stimulatory, effect on uterine NO production [20]. Therefore, we suggest that the dose of estrogen or the changes in serum levels of estrogens in different stages of pregnancy (gestational age) may affect NO production in the uterus in response to apelin /APJ system and consequently the degree of its utero -relaxant effect.

During pregnancy, when progesterone levels are elevated, increased NOS expression and NO production in the uterus may inhibit uterine contractility and help maintain uterine quiescence [21]. This may explain at least in part, the marked utero-relaxant effect of apelin in early pregnancy.

Many studies suggest that increased PG production at term may down-regulate uterine NO production and therefore facilitate labor. A decrease in progesterone levels and an increase in estradiol levels at term could increase cyclooxygenase-2 (COX-2) expression. Therefore, the elevated PG production could down-regulate NOS and NO production**.** This could lead to initiation of labor, similar to that demonstrated in preterm labor [22]. And it could explain, at least part , the decrease in apelin induced utero-relaxant effect late in pregnancy.

The major mechanism of action of apelin induced NO is via activation of soluble guanylyl cyclase with subsequent formation and increase in the level of cGMP . However NO may act through cGMP indepndent pathway i.e (through potassium channels). Hence, the inhibition of these channels, mainly Ca^{2+} sensitive K^+ channels decreases the inhibitory effect of NO supporting the importance of these channels, particularly at mid gestation [15].

In myometrium, K^+ channels are an essential component of the mechanism that allows for adaptation of the gravid uterus to increases in stretch and intrauterine pressure, several types of K^+ channels have been identified in the myometrium. The most abundant and well-studied include the large-conductance Ca^{2+}- and voltage-sensitive K^+ channel (BKCa channel), the ATP-sensitive K^+ channel (K_{ATP}) and small conductance calcium-sensitive potassium channels (SK) [23].

Therefore, we focused in the present study on some types of K^+ channels to clarify whether these channels are involved in the mechanism of action of apelin or not. The present study revealed that apelin utero relaxant effect was partially but significantly attenuated by apamin. This finding may indicate that the SK channels may be involved at least in part in the utero relaxant effect of apelin.

SK channels generate a hyperpolarizing current in excitable cells following action potential generation, and thus may induce relaxation of smooth muscle [24]. Also, SK channels appeared as critical regulators of myometrial contractility during gestation and

labor. In human non-pregnant and pregnant myometrium, apamin, an inhibitor of SK channels, attenuated relaxation induced by NO[13].

Many studies have demonstrated that SK3 over expression was associated with compromised labor possibly due to inefficient labor contractions and showed that it was accompanied with depression of phasic contractions in mouse uterus by limiting the influx of Ca^{2+} [25] .

However, BKCa channel may be also involved in the utero relaxant effect of apelin . They are abundant in uterine smooth muscle and have a significant repolarizing current. Relatively few BKCa channels need to be activated to produce uterine relaxation [14].

Noble et al. [26] showed that all SK channel isoforms (SK1–3) are expressed and translated throughout pregnancy in pregnant rat myometrium and they contribute more to quiescence than BKCa channels.

Moreover , the present study showed that the apelin utero relaxant effect was partially but significantly decreased in the presence of K_{ATP} channel blocker (Glibenclamide). This observation indicate that K_{ATP} channels may be involved , at least in part, in the apelin utero relaxant effect.

K_{ATP} channels have an important role in regulation of uterine quiescence during pregnancy. Low expression of the K_{ATP} channels at the end of gestation may facilitate enhanced excitability and contractility in the rat myometrium [27].

The mechanism/s of the relaxant effect of apelin proved in the present study are supported by the study of Yang et al. [28] who demonstrated that apelin binds with APJ leading to increased Ca^{2+} influx that will lead to activation of NOS , phospholipase A_2 and increased release of endothelial derived hyperpolarizing factor (EDHF) which induces smooth muscle relaxation .

The effect of apelin at late pregnancy was significantly less at early pregnancy which may be attributed to the hormonal dependent decrease in activity and/or expression of both NOS and K^+ channel as it was previously discussed. It could be also explained by the decrease in the number of APJ that may be accounted for by either the decrease in the synthesis of APJ [29], and or down regulation [30] and internalization of APJ [31].

CONCLUSION

Apelin has a potent dose dependent relaxant effect. This may be attributed to aplein/APJ-induced increase in activity and/or over expression of both NOS and K^+ channels which may be produced by hormonal changes occurring during pregnancy. Hence, apelin may be a promising tocolytic agent that can be used to prevent abortion and preterm labour and to treat spasmodic dysmenorrhea.

RECOMMENDATION

Further studies are required to investigate the effects of apelin on uterine reactivity of obese, diabetic and pre-eclamptic pregnant women.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Funding information: None declared

REFERENCES

[1] Lee D K, Cheng R, Nguyen T et al.Characterization of apelin, the ligand for the APJ receptor. *J. of neurochemistry*, 2000 ;*74*(1) : 34-41.

[2] O'Carroll A. M, Lolait S. J and Pope G. R. The apelin receptor APJ: journey from an orphan to a multifaceted regulator of homeostasis. *J. of Endocrinology*, 2013 ; *219*(1) :R13-R35.

[3] Chapman N. A, Dupré D. J and Rainey J K. The apelin receptor: physiology, pathology, cell signalling, and ligand modulation of a peptide-activated class A GPCR. *Biochemistry and cell biology*, 2014 ;*92*(6) :431-440.

[4] Alsaif S, Mumtaz S and Wray S. A. short review of adipokines, smooth muscle and uterine contractility. *Life Sci* , 2015 ; 125 :2–8.

[5] Hehir M P and Morrison J J . The adipokine apelin and human uterine contractility. *Am. J. Obstet. Gynecol* 2012 ; 206, 359.e1-359.e5

[6] Kacar E , Ercan Z and Kutlu S : The effects of apelin on myometrium contractions in pregnant rats. *Cellular and molecular biology (Noisy-le-Grand, France)* 2018; *64*(11) :74-79.

[7] Sharma N, Mehta A A and Goyal R K :Evidence for alpha 2-adrenoceptor agonist activity of minoxidil. J. Pharm. Pharmacol

1997 ;49 :935–7.

[8] Scorza Barcellona P , Fanelli O and Campana A. Teratological study of etoperidone in the rat and rabbit. *Toxicology* ,1977 ; 8 :87–94

[9] Klukovits A , Gáspár R and Falkay G . Functional and histochemical characterization of a uterine adrenergic denervation process in pregnant rats. Biol. Reprod , 2002 ; 67 :1013–7.

[10] Sladek S. M and Roberts J. M .Nitric oxide synthase activity in the gravid rat uterus decreases a day before the onset of parturition. Am. J. Obstet. Gynecol , 1996 ; 7 :166– 175

[11] Novaro V, Rettori V and De Gimeno, M A. F: Interaction between uterine PGE and PGF2α production and the nitridergic system during embryonic implantation in the rat.Prostaglandins , 1996 ; *51*(6) : 363-376.

[12] Yildirim K , Sarioglu Y and Yildirim S: Inhibitor effect of omeprazole in isolated human myometrial smooth muscle. *Life Sci.* 2001 ; 69 :435–42.

[13] Modzelewska B, Kostrzewska A and Batra S: Apamin inhibits NO-induced relaxation of the spontaneous contractile activity of the myometrium from non-pregnant women. Reprod. Biol. Endocrinol. 2003; 1:1–8.

[14] Sadlonova V, Franova S and Sadlonova J. Participation of $BKCa^{2+}$ and K_{ATP} potassium ion channels in the contractility of human term pregnant myometrium in in vitro conditions. J. of Obstetrics and Gynaecology Research , 2011; 37(3) :215-221.

[15] Okawa T, Asano K and Gafield R E .Expression of iNOS mRNA and inhibitory effect of NO on uterine contractile activity are determined by local rather than systemic factors of pregnancy. J.of pharmacological sciences, 2004; 0407080011-0407080011.

[16] Melgar-Lesmes P, Pauta M, Reichenbach V et al. Hypoxia and proinflammatory factors upregulate apelin receptor expression in human stellate cells and hepatocytes. *Gut*, 2011; *60*(10) :1404-1411.

[17] Maul H , Longo M & Garfield, R. E. Nitric oxide and its role during pregnancy: from ovulation to delivery. *Current pharmaceutical design*, 2003 ; *9*(5) :359-380.

[18] Figueroa J. P and Massmann G. A. Estrogen increases nitric oxide synthase activity in the uterus of nonpregnant sheep. *Am.J. Obstet. Gynecol*, 1995; 173:1539–45

[19] Magness R. R, Shaw C E and Bird I. M . Endothelial vasodilator production by uterine and systemic arteries. II. Pregnancy effects on NO synthase expression. *Am. J. Physiol. Circ. Physiol* ,1997; 272 : H1730–H1740.

[20] Yallampalli C, Byam-Smith M and Garfield, R. E.. Steroid hormones modulate the production of nitric oxide and cGMP in the rat uterus. *Endocrinology*, 1994;*134*(4), 1971-1974

[21] Yallampalli C, Dong Y L and Fang L. Role and regulation of nitric oxide in the uterus during pregnancy and parturition. *J. Soc. Gynecol. Investig*, 1998; 5 :58–67.

[22] Franchi A. M, Chaud M and Gimeno, M.. Role of nitric oxide in eicosanoid synthesis and uterine motility in estrogen-treated rat uteri. *Proceedings of the National Academy of Sciences*, 1994 *;91*(2) :539-543.

[23] Brainard, A. M., Korovkina, V. P and England, S. K. Potassium channels and uterine function. In *Seminars in cell & developmental biology* , Academic Press , 2007; 18 (3) :332-339.

[24] Shmukler B E, Bond C T and Alper S L. Structure and complex transcription pattern of the mouse SK1 KCa channel gene, KCNN1. Biochimica et Biophysica Acta (BBA)-Gene Structure and Expression, 2001; *1518*(1-2), 36-46.

[25] Brown A, Cornwell T , Korniyenko I and Taylor M S. Myometrial expression of small conductance Ca^{2+}-activated K^+ channels depresses phasic uterine contraction. American J. of Physiology-Cell Physiology , 2007; *292*(2) : C832-C840.

[26] Noble K , Floyd R and Wray S : Distribution, expression and functional effects of small conductance Ca^{2+} activated potassium (SK) channels in rat myometrium. *Cell calcium* , 2010; *47*(1) : 47-54.

[27] Lovasz N , Koncz A and Falkay G: ATP-sensitive potassium channels modulate in vitro tocolytic effects of β2-AR agonists on uterine muscle rings in rats in early but not in late pregnancy. Croat. Med. *J.* 2015 ; 56: 114–118.

[28] Yang P, Maguire J. J and Davenport A. P. Apelin , Elabela / Toddler , and biased agonists as novel therapeutic agents in the cardiovascular system. *Trends Pharmacol. Sci*, 2015 ;36 :560–567.

[29] Wu Y , Wang X and Bai B. Temporal expression of apelin/apelin receptor in ischemic stroke and its therapeutic

potential. *Frontiers in molecular neuroscience*, 2017 ; *10*: 1

[30] Iwanaga Y, Kihara Y and Kita T. Down-regulation of cardiac apelin system in hypertrophied and failing hearts. *J. Mol. Cell. Cardiol*, 2006 ; 41: 798–806

[31] Pope G R, Tilve Sand O'Carroll A M. Agonist-induced internalization and desensitization of the apelin receptor. *Mol. Cell. Endocrinol*, 2016 ; 437 :108–119.

To Cite This Article: Ali KA, Shreif WM, Kamelia IA, Somya EM. Apelin Induced Modulation of Uterine Contractility in Adult Albino Rats and its Possible Mechanism/s of Action.ZUMJ 2020;26(1);174-185.DOi: 10.21608/zumj.2019.11148.1157