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Apelin Induced Modulation of Uterine Contractility in Adult Albino Rats and its Possible Mechanisms of Action

Mansour SW*, Asalah AK, Attia KI and El Sayed Mohammed S
Department of Physiology, Faculty of Medicine, Zagazig University, Egypt

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, brain, 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 is not fully investigated despite some author report that NO may be involved.

Aim of the study: This study was designed to demonstrate the *in vitro* effects of apelin on spontaneous contraction of pregnant and non-pregnant rat uterus and to investigate its possible mechanism/s of action.

Material and methods: Sixty 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 organ bath fluid) on spontaneous contractile activity of isolated uterine strips were studied. Also, the effect of apelin on uterine strips isolated from pregnant rats (100 nmol/L) was investigated in the presence of L-NAME, apamin and Glibenclamide.

Results: Apelin exerted a significant dose dependent reduction in frequency and amplitude of spontaneous uterine contraction. This utero-relaxant effect of apelin was significantly more potent on day 6 of gestation than that in both non pregnant rats and pregnant rats on day 19 of gestation. Apelin induced utero-relaxant effect was significantly and nearly completely abolished in the presence of L-NAME, but it was significantly and partially decreased in the presence of small conductance-Ca^{2+} activated K^+ channel blocker (Apamin) and ATP sensitive K+ channel blocker (Glibenclamide).

Conclusion: 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.

Keywords: Apelin; Uterine contractility; Pregnancy

Introduction

Apelin is an adipokine and the endogenous ligand for APJ, an angiotensin-1-like receptor. It has been isolated from the bovine stomach [1]. 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 [2].

Apelin and APJ were detected in various tissues and organs such as stomach, brain, heart, lung, uterus, ovary [3] and produced in pregnant and lactating breast [4], high levels were identified in the placenta suggesting a possible placental origin of apelin in pregnancy [5]. Apelin acts via apelin receptor (APJ) to mediate effects on the cardiovascular system [6] fluid homeostasis [7], glucose metabolism [8], and food intake [9], influencing not only cyclic AMP production but also protein kinase c(PKC), phosphatidyl inositol 3 kinase (PI3K), protein kinase B (Akt), S6 ribosomal protein kinase (p70S6K), extracellular regulated kinase (ERK) [10,11] and cytoplasmic Ca^{2+} concentration [12]. Apelin exerts an inotropic effect on the heart and simultaneously elicits vasodilatation in the peripheral circulation [13]. Regulation of the frequency and intensity of uterine contraction is necessary for several functions of the female reproductive tract, including implantation of the embryo, transport of sperm for fertilization, and formation of contractile waves associated with the menstrual cycle [14]. Uterine contraction during pregnancy and labor may be subjected to regulation by metabolic factors as secretory products of adipose tissue [15]. The myometrial smooth muscle, as a nonvascular smooth muscle, appears to respond to apelin exposure in a similar way as does vascular smooth muscle [16]. There are contradictory reports about the effect of apelin on uterine contractility. Hehir and Morrison [17] observed that apelin exerts an inhibitory effect on human myometrial contractility *in vitro*, in tissue obtained during pregnancy. In contrast, Kacar et al. [18] reported that apelin induces myometrial contractions. Because of these contradictory reports, the present study was designed to demonstrate the effect of apelin on spontaneous contraction of uterine strips isolated from both pregnant and non-pregnant rats and the possible involvement of Nitric Oxide (NO), and small conductance Ca^{2+} activated K^+ channels and ATP sensitive K^+ in apelin induced effect.

Materials and Methods

Material (Animals)

Sixty healthy adult albino rats (48 female rats and 12 male rats) were obtained from the laboratory animals' farm unit Faculty of Veterinary Medicine, Zagazig University, with an average weight, 180-200 grams. The animals were kept in steel wire cages (6/cage) under hygienic conditions and kept on the diet which consisted of mixed commercial rat laboratory chow and supplied in separate clean containers. Animals had free access to water and kept at room temperature. All animals were bred in the animal house. The rats were accommodated to laboratory conditions for two weeks before the experiments going on. The male rats were used for induction of pregnancy. The experimental protocol was approved by physiology

***Corresponding author:** Mansour SW, Professor, Department of Physiology, Faculty of Medicine, Zagazig University, Egypt, Tel: +201115491222; E-mail: drsherif36@hotmail.com

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department and by local medical ethics committee in faculty of medicine of Zagazig University (Institutional Review Board, IRB).

Groups: The animals were randomly divided into the following groups (12 for each):

Male group (n=12): used for induction of pregnancy of female rats

Female groups (n=48): The animals randomly divided in to

Group 1(n=12): Healthy adult female rats in estrous phase (non-pregnant)

Group 2 (n=24): Healthy adult pregnant female rats which further subdivided into.

Group 2a (n=12): Early pregnant at day 6 of gestation.

Group 2b (n=12): Late pregnant at day 19 of gestation.

Group 3 (n=12): 1st day post-partum.

Drugs and chemicals:

Apelin-13 trifluoroacetate salt: (Sigma- Aldrich co. USA).

NG-nitro-L-arginine methyl ester (L- NAME), nitric oxide synthase inhibitor: (Sigma- Aldrich co. USA).

Apamin, small conductance Ca^{2+} activated K+ channels blocker: (Sigma- Aldrich co. USA).

Glibenclamide, ATP sensitive K+ channel blocker.

NB: The previous chemicals were dissolved in distilled water.

De Jalone solution gm/2 L (NaCl, 18; KCl, 0.84; Glucose, 1; Na HCO3,1; CaCl2,0.4). The pH of this solution was 7.4 and it was bubbled with Carbogen (95%O2 and 5% CO2) to be used as a bath fluid for isolated uterine strips [19]. All the chemicals used for preparing De-Jalone solution were purchased from El Nasr Pharmaceutical Chemicals CO. Abu Zaabal, Egypt.

Methods

Preparation of the non-pregnant group

The non-pregnant female rats were prepared with subcutaneous injection of diethylstilbestrol (0.5 mg/kg) 24 h before the experiment started for sensitization of the uterine smooth muscle [20].

Timed- pregnant group

Determination of the first day of pregnancy: Vaginal smears taken from the female rats were examined daily by using light microscope to ensure that they were in regular estrus cycle. The estrus phase of the estrus cycle was detected by the presence of cornified epithelial cells which increase in number and eventually predominate as the estrus progresses [21].

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 [22]. 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 [23].

Isolated uterine tissue protocol

Rats were sacrificed under light ether anaethesia in the estrus phase in the non-pregnant group (group 1), on day 6 of gestation (group 2a, Early pregnant), on day 19 of gestation (group 2b, Late pregnant) and on 1st day post -partum (group 3) 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 × 1.3 cm in length [24]. The strips were mounted in De Jalone solution of pH 7.4 at temperature of 37°C, aerated with a mixture of 95% O_2 and 5% CO_2 in the organ bath (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 to a thread. The preparation required approximately 1 hour to equilibrate after dissection, isometric uterine contractions were recorded by a transducer (Letica Scientific Instruments) connected to Bridge Amp amplifiers (AD Instruments, Australia) with 4 channel data acquisition system (Power Lab/4/20, AD Instruments) connected to computer, data were saved electronically and quantified using the program Chart 7.2.

The strips were bathed with De-Jalone solution. After spontaneous activity became regular various agents were added. 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 was added in three separate doses- 1, 10 and 100 nmol/L organ bath fluid [17] to organ baths containing uterine strips isolated from;

- 12 non-pregnant adult female rats
- 12 rats on day 6 of gestation
- 12 rats on day 19 of gestation
- 12 rats on 1st day postpartum.

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:

- NG-nitro-L-arginine methyl ester (L-NAME) (3 × 10-5 mol/L) [25]
- Apamin (10-8 mol/L) [26]
- Glibenclamide (10-6 mol/L) [27].

The isolated uterine 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 by the strips after the addition of each dose of apelin alone or apelin in the presence of different types of chemicals, were quantitated and expressed as the percentage of reduction in the amplitude or the frequency generated during the spontaneous contractile activity before the addition of these agents (the control).

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

The effect of different doses of apelin (1, 10 and 100 nmol/L organ bath fluid) on spontaneous contractility of uterine strips isolated from non-pregnant, pregnant rats on day 6, 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 (Tables 1a and 1b; Figure 1).

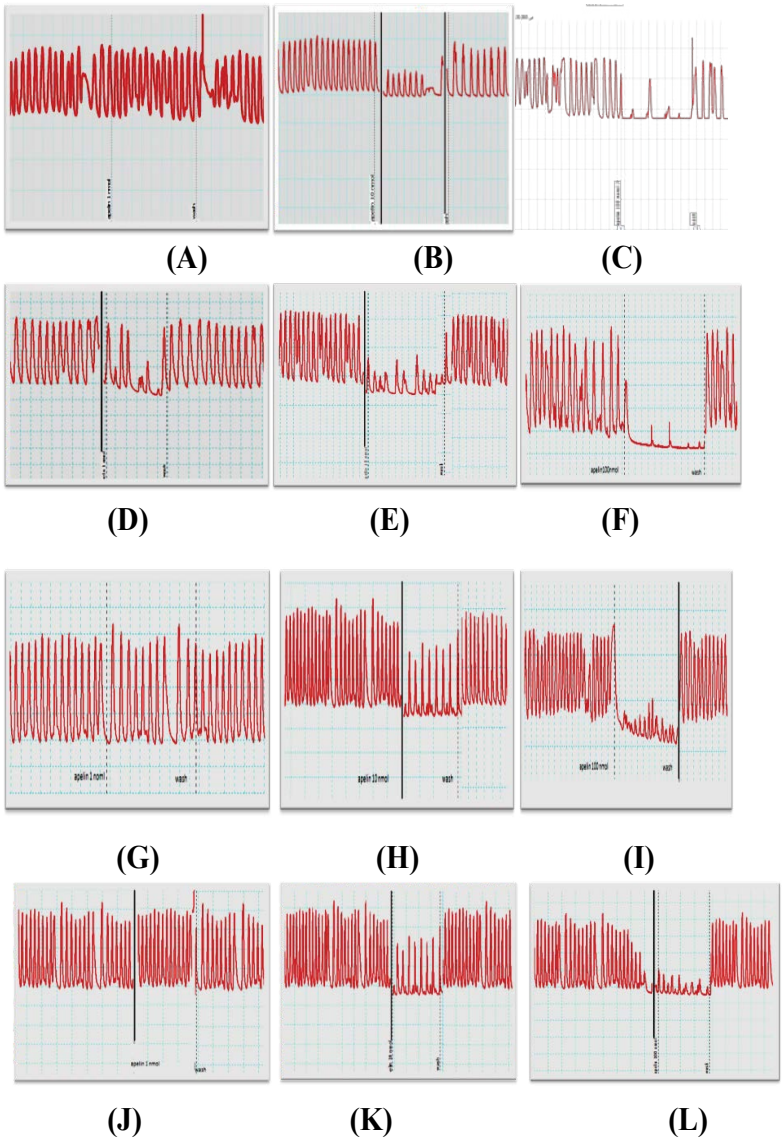
Amplitude (mm)									
	Group 1(non-pregnant)								
	1 nmol/L organ bath fluid			10 nmol/L organ bath fluid			100nmol/L organ bath fluid		
	Before	After	% of change	Before	After	% of change	Before	After	% of change
$\bar{x}$	6.25	5.75	10	7.08	3.83	45.97	6.5	1.08	82
±SD	1.38	1.21	10.44	1.44	0.93	6.93	1.24	0.79	13.09
"t"	3.31			14.93			17.31		
P	NS (p > 0.05)			(p<0.05			(p<0.05)		
r	0.948 (p<0.001)								
Group 2a (Early pregnant at day 6 of gestation)									
	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
$\bar{x}$	16.5	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"	14.83 (p<0.05)			24.2 (p<0.01)			40.04 (p<0.001)		
P									
R	0.961(p<0.001)								
Group 2b (Late pregnant at 19 day of gestation)									
	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
$\bar{x}$	17.75	15.91	10.2	18.4	11	40	17.25	4.6	72.84
±SD	2	1.97	4.15	2.5	1.8	6.09	1.95	1.07	6.2
"t"	8.84 NS (p > 0.05)			16.4214.83 (p<0.05)			23.79(p<0.001)		
P									
r	0.979(p<0.001)								
Group 3(1st day post-partum)									
	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
$\bar{x}$	11.16	10.16	9.4	9.58	5.6	40.4	9	1.6	81.16
±SD	2.03	2.28	8.68	1.37	0.77	7.27	1.2	1.3	14.29
"t"	4.06 NS (p > 0.05)			11.65 (p<0.05)			13.32 (p<0.001)		
R	0.943(p<0.001)								

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

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
$\bar{x}$	16	15.25	4.92	17	8.5	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"	3.44			21.31			26.69		
p	(p<0.05)			(p<0.05)			(p<0.05)		
R	0.951(p<0.001)								
Group 2a (Early pregnant at day 6 of gestation)									
	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
$\bar{x}$	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"	13.4			25.12			39.14		
P	(p<0.05)			(p<0.001)			(p<0.001)		
R	0.936(p<0.001)								
Group 2b (Late pregnant at 19 day of gestation)									
	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
$\bar{x}$	18.4	17.5	5.1	18	12	34	18.75	4.33	72.84
±SD	1.44	1.67	4.5	2	1.8	4.76	2.09	0.77	6.23
"t"	4NS			28.14			28.14		
P	(p > 0.05)			(p<0.05)			(p<0.001)		
R	0.983(p<0.001)								
Group 3 (1st day post-partum)									
	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
$\bar{x}$	18.3	17.6	3.46	18.25	11.66	36.18	18.25	4	78.35
±SD	1.43	1.15	4	1.4	1.6	5.68	1.4	2	10.65
"t"	2.96NS			22.89			27.97		
P	(p > 0.05)			(p<0.05)			(p<0.001)		
R	0.972(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



NB: r=correlation between dose of apelin (nmol/Lorgan bath fluid) and percentage of reduction (±SD) of amplitude and frequency of contraction of uterine strips.

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)

This relaxant effect was found to be dose dependent because there was a significant positive correlation between the relaxant effect and the doses used (r=0.948 for amplitude and 0.951 for frequency in non-pregnant, r=0.961 for amplitude and 0.936 for frequency in pregnant rats on day 6 of gestation and r=0.979 for amplitude and 0.983 for frequency in pregnant rats on day 19 of gestation and r=0.943 for amplitude 0.972 for frequency in 1st day post-partum).

Table 2 and, Figure 2a and 2b show a comparison between the percentages of reduction ($\bar{X} \pm SD$) of amplitude and frequency of spontaneous contraction of uterine strips isolated from non -pregnant rats, pregnant rats on day 6 and pregnant rats on day 19 of gestation in the presence of different doses of apelin. It was observed that, the utero-relaxant effect of apelin was significantly higher in uterine stripe isolated from the group of pregnant rats on day 6 of gestation when compared with both non pregnant group and group of pregnant rats on day 19 of gestation. However, a non-significant difference in the utero-relaxant effect of apelin was observed when comparing the non-pregnant group with the pregnant group on day 19 of gestation.

Table 3, Figures 3 and 4 show the effect of apelin (100 nmol/L organ bath fluid) on spontaneous contractility of uterine strips isolated

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	46	60	40	40.4	82	88.19	72.84	81.16
±SD	10.44	5.15	4.15	8.68	6.93	8.28	6.09	7.27	13.1	9.33	6.2	14.29
F	5.85 (p< 0.001)				20.6 (p<0.001)				8.31 (p< 0.001)			
P of LSD VS group 1		< 0.001	NS	NS		< 0.001	NS	NS		< 0.05		
Percentage of reduction in frequency (cycle /20 min)												
$\bar{x}$	4.92	19.83	5.1	3.46	48.9	69.1	34	36.18	78.3	91.47	72.84	78.35
±SD	4.91	5.15	4.5	4	5.98	4.3	4.76	5.68	14.6	6.34	6.23	10.65
F	N\A				N\A				N\A			
P of LSD VS group 1		< 0.001	NS	NS		< 0.001	NS	NS		< 0.05		

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

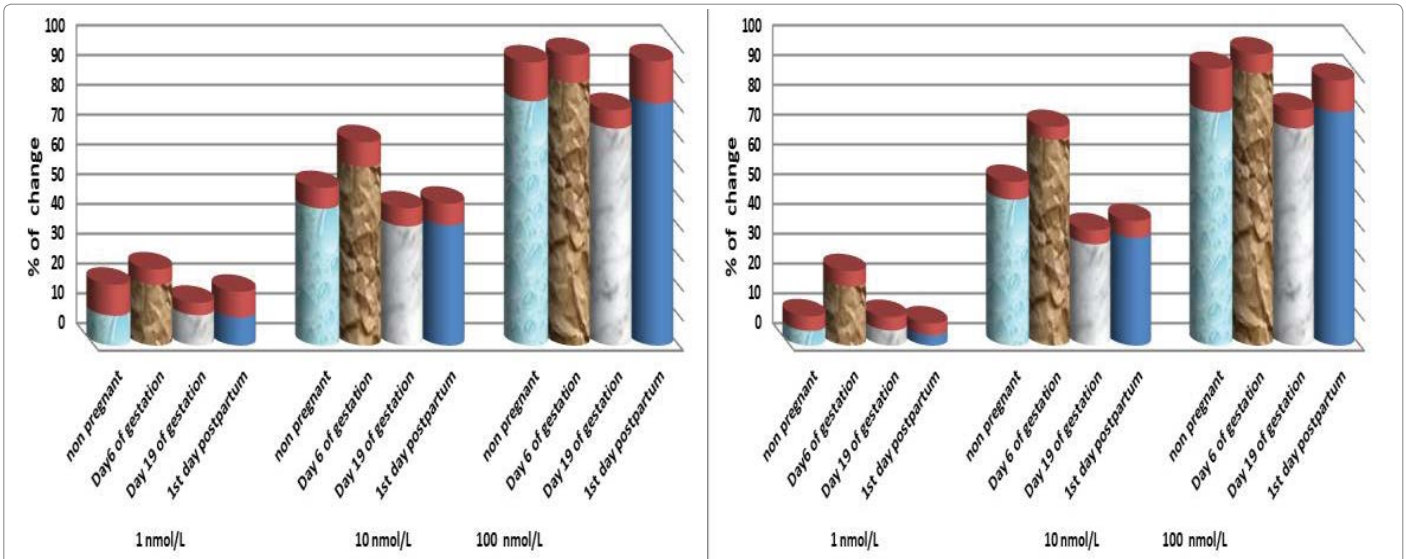


Figure 2a and 2b: The percentages of reduction ($\bar{x} \pm SD$) of amplitude (a) and frequency (b) of spontaneous contraction of uterine strips isolated from non -pregnant rats, rats on day 6 , day 19 of gestation and 1st day post-partum in the presence of different doses of apelin (1st dose 1, 2nd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

N =6	Amplitude (mm)								
	L-NAME + Apelin			Apamin& Apelin			Glibenclamide & Apelin		
	Before	After	% of reduction	Before	After	% of reduction	Before	After	% of reduction
$\bar{x}$	15.3	13.16	14.13	15.3	6	59.64	15.3	4.16	71.5
±Δ	2.16	2.04	4.5	2.16	0.89	11.34	2.16	1.16	11.5
"t"	7.05 (p<0.001)			8.15(p<0.001)			9.5(p<0.001)		
Frequency (cycle /20 min)									
$\bar{x}$	16.6	14.3	14.12	16.6	7	41.5	16.6	12.6	74.7
±Δ	3.5	3.3	5.5	3.5	1.7	5.6	3.5	2.7	4.8
"t"	5.53(p<0.001)			10.5 (p<0.001)			1.4 (p<0.001)		

Table 3: Effect of apelin (100 nmol/L organ bath fluid) on the amplitude (mm) and frequency of spontaneous contraction of uterine strips isolated from pregnant rats (day 6) before and after incubation with apelin (100 nmol/L), L-NAME ($3x10^{-5}$ mol/L) , Apamin (10^{-8} mol/L) and Glibenclamide (**(**10^{-6} mol/L).

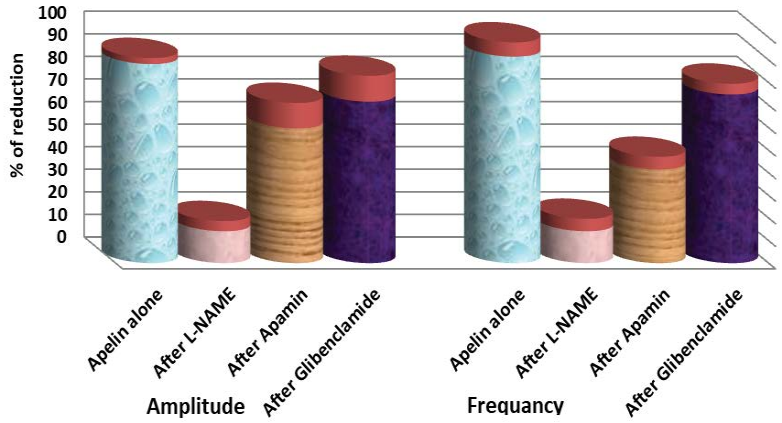


Figure 3: The percentages of reduction ($\bar{x} \pm SD$) of amplitude (a) and frequency (b) of contraction produced by addition of apelin (100 nmol/L organ bath fluid) to uterine strips isolated from pregnant rats (day 6) before and after incubation with L-NAME (3 x 10^{-5} mol/L), Apamin (10^{-8} mol/L)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

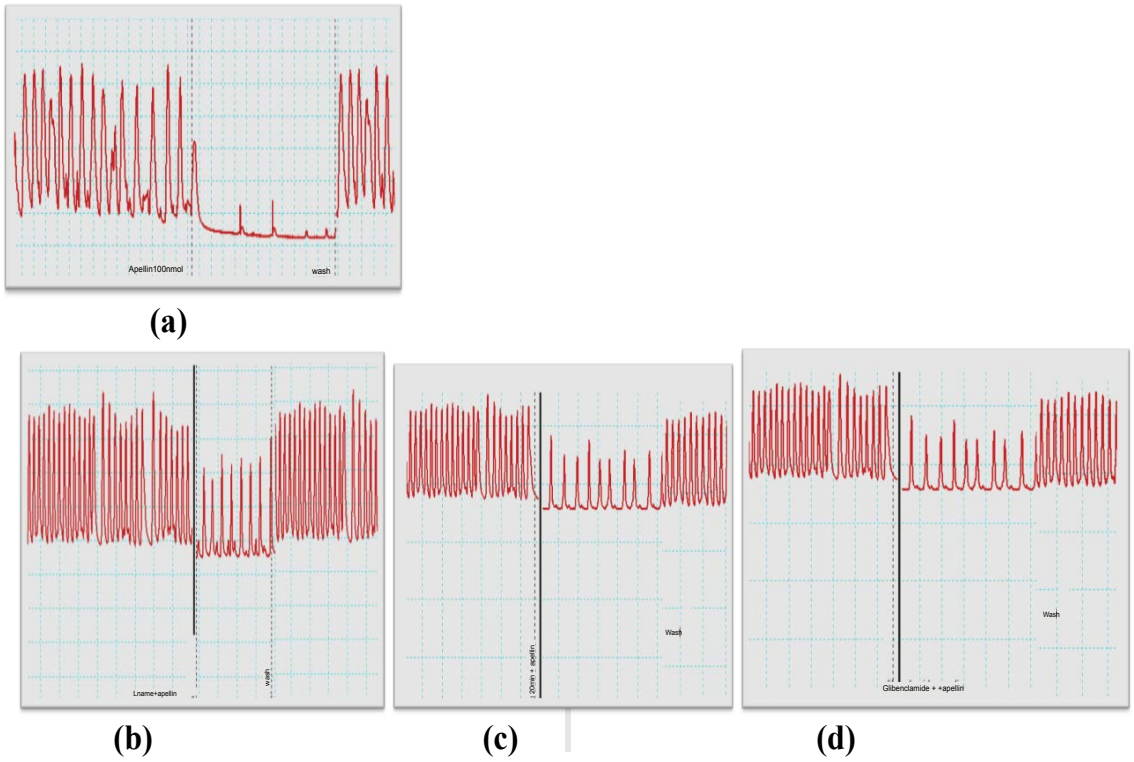


Figure 4: Representative recordings demonstrating the effect of apelin (100 nmol/L organ bath fluid) on spontaneous contractility of uterine strips isolated from pregnant rats on(day 6)of gestation in the presence of, L-NAME, (3x10^{-5} mol/L), Apamin, (10^{-8} mol/L) and Glibenclamide, (10^{-6} mol/L).

from pregnant rats on day 6 of gestation in the presence of nitric oxide synthase inhibitor L-NAME, (3 × 10^{-5}mol/L), small conductance Ca^{2+} activated K^+ channel blocker Apamin, (10^{-8} mol/L) and ATP sensitive K^+ channels blocker Glibenclamide, (10^{-6} mol/L). It was found that the utero-relaxant effect of apelin was significantly and nearly completely abolished in the presence of L-NAME, but it was partially blocked in the presence of Apamin and Glibenclamide

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 and non-pregnant rats. This relaxant effect was dose dependent and was observed at relatively low concentrations for *in vitro* experiments (in nanomolar range). The apelin utero relaxant effect encountered in the present study is in agreement with the results of Hehir and Morrison [17], who studied the effect of apelin on human uterine strips isolated from pregnant human 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. This relaxant effect was observed at relatively low concentrations for

in vitro experiments (nanomolar range). However, our results are at variance with those of Kacar et al. [18] 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 plays a role on uterine contraction at birth in rats facilitating parturition. The utero-relaxant effect of apelin encountered in the present study may be attributed to apelin induced release of NO which may be mediated by increased activity of NOS after binding of apelin to its receptors APJ. This finding was proved in the present study 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 nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME).

The pregnant uterus is relatively relaxed and quiet while enlarging to accommodate the developing fetus. NO has a relaxant effect on myometrium, it is thought that 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) [28].

The apelin-induced utero relaxant effect observed in the present study 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. Progesterone regulates NOS and NO production during gestation. Not only progesterone affect the generation of NO, but it may also up-regulate the effects of NO [29]. Estrogen-induced increases in NOS activity were reported in the uteri of sheep and rats [30]. Increased vaso-relaxation and increased expression and activity of vascular NOS were reported to be associated with estradiol levels [31], a biphasic effect of estrogens on constitutive NOS activity was reported with stimulatory effects in lower doses and inhibitory effects in higher doses. 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 [32]. 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 [33]. 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 [34] and 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 independent 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 [28]. 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 (KATP), the Shaker-like voltage-gated K+ channels (KV), and small conductance calcium-sensitive potassium channels (SK). The variety of K^+ channels in this tissue reflects the multiplicity and complexity of the mechanisms involved in the regulation of uterine tonus [35].

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 small conductance Ca^{2+} sensitive potassium ion channel blocker (apamin). This finding may indicate that the small conductance Ca^{2+} sensitive K channels may be involved at least in part in the utero relaxant effect of apelin.

Small-conductance Ca^{2+} sensitive and voltage-insensitive potassium channels (SK) generate a hyperpolarizing current in excitable cells following action potential generation, and thus may induce relaxation of smooth muscle [36]. 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 [26]. 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+} [37]. However, the big conductance Ca^{2+} sensitive K^+ channel may be also involved in the utero relaxant effect of apelin. The BKCa channels are large conductance, voltage$^-$ and Ca^{2+} sensitive K^+ channels. 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 [35].

Noble et al. [38] showed that all small conductance Ca^{2+} activated K^+ (SK) channel isoforms (SK1–3) are expressed and translated throughout pregnancy in pregnant rat myometrium and they contribute more to quiescence than large conductance Ca^{2+} activated K^+ (BK) channels. Due to a constitutive association with calmodulin, SK3 channels are highly sensitive to changes in cytosolic Ca^{2+} levels [39,40] and are thus capable of exerting abrupt negative feedback regulation of intracellular Ca^{2+} [37]. 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. ATP-sensitive inward rectifying potassium channels (K_{ATP}) have an important role in regulation of uterine quiescence during pregnancy. The inward rectifier K^+ channel family Kir 6 comprises the potassium channel component of the K_{ATP}, while the sulfonylurea receptor (SUR) is responsible for the ATP sensitivity, pharmacological properties, and trafficking of this channel [41].

Low expression of the KATP channels at the end of gestation may facilitate enhanced excitability and contractility in the rat myometrium [42]. APJ signaling via NOS/NO system proved in the present study was supported by other studies, in anaesthetized rats where the hypotensive action of apelin was abolished by the NOS inhibitor L-NG-

nitroarginine methyl ester (L-NAME [43]). Similar findings have been observed in mice, where apelin-induced phosphorylation of endothelial NOS (eNOS) was observed in isolated mouse endothelial cells [6]. In the isolated rat aorta, apelin stimulates the transport of L-arginine and enhances the activity of e NOS to stimulate the production of nitric oxide, while post-infarct treatment of rats with (Pyr1) apelin-13 significantly increases serum nitric oxide levels [44]. Apelin has also been involved in signaling via NOS in the aortic ring of diabetic mice and in the control of glucose metabolism in mice, as validated by studies carried out with a NOS inhibitor and e NOS knockout (KO) mice [45].

Also, The mechanism/s of the relaxant effect of apelin proved in the present study are supported by the study of Yang et al. [46] who demonstrated that apelin binds with G protein coupled receptors (APJ) leading to increased Ca2+ influx that will lead to activation of NOS , phospholipase A_2 and increased release of endothelial derived hyperpolarizing factor (EDHF). Increased activity of NOS leads to enhanced synthesis of NO which induces smooth muscle relaxation either by increased K^+ efflux (hyperpolarization) of the cell membrane or by activating of Gs/cGMP pathway which in turn activate PKG that induces relaxation by decreasing intracellular Ca^{2+} level in the cytoplasm of smooth muscle and de phosphorylation of myosin light chain. On the other hand, The authors postulated the apelin –induced activation of PLA_2 increases formation of PGI2 from Arachodinic Acid, PGI_2 (prostacyclin) binds to GPCR activating AC/PKA pathway that lead to increased formation of cAMP that reduces Ca^{2+} sensitivity of contractile system and increases K^+ efflux. Finally, the authors stated that the apelin induced increase release of EDHF is associated with hyperpolarization and relaxation of the smooth muscle via activation of K^+ ion channels [46].

The present study revealed that the *in vitro* utero-relaxant effect of apelin observed at late pregnancy was significantly less than that observed at early pregnancy. This significant deference 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 apelin receptors that may be accounted for by either the decrease in the synthesis of apelin receptors [47], and or down regulation [48,49] and internalization of these receptors.

Conclusion

Apelin has a potent dose dependent relaxant effect particularly on the uterine strips isolated from both non pregnant and early pregnant rats. This potent utero-relaxant effect 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 during pregnancy of obese, diabetic and pre-eclampsia.

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