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Physiology

EFFECT OF ABSCISIC ACID ON GASTRIC MOTILITY IN NORMAL AND DIABETIC RATS

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ABSTRACT Abscisic acid (ABA) is emerging as a new important player in glucose homeostasis. Here, we study its effect on gastric motility in normal and diabetic rats. Forty eight Sprague-Dawley rats were divided into two groups: group I, (Control, n=24), which was subdivided into: subgroup (I a) to determine the effect of ABA in different doses, after adding autonomic drugs (I b), after adding Ca^{+2} channel blockers (I c), after adding L-NAME (I d), after adding Na^{+} channel blocker (I e), after adding PPAR -γ antagonist (I f), and group II (Diabetic, n=24), which was subdivided into: subgroup (II a) to determine the effect of ABA in different doses, after adding autonomic drugs (II b), after adding Ca^{+2} channel blockers (II c), after adding L-NAME (II d), after adding Na^{+} channel blocker (II e), after adding PPAR -γ antagonist (II f). ABA increased basal gastric motility with higher response in diabetic rats with increased expression of LANCL2 receptors. Also, ABA had excitatory effect after blocking of autonomic receptors. Ca^{2+} channel blockers diminished the excitatory effect of ABA. Also, ABA led to a significant excitatory effect after adding L-NAME. ABA increased gastric motility after adding lidocaine. The excitatory effect of ABA can be abolished after adding PPAR –γ antagonist.

KEYWORDS : Abscisic acid; LANCL2 receptors; gastric motility

1. INTRODUCTION

Abscisic acid (ABA) is a phytohormone regulating fundamental physiological functions in plants[1]. Recently, ABA has been identified in mammalian plasma and several cell types such as human granulocytes and pancreatic β cells[2].

ABA, at Nanomolar concentrations, enhances insulin secretion in response to glucose in rat insulinoma cells, human and murine pancreatic islets [3]. Also, high glucose concentrations stimulate ABA production and release from human and rat pancreatic β cells, suggesting that ABA is an endogenous activator of insulin release. ABA is also produced by inflammatory cells, monocytes and granulocytes, indicates that this acid may participate to the complex network of cytokine signals exchanged between pancreatic β cells and inflammatory cells, which is progressively recognized as a fundamental mechanism in the development of metabolic syndrome and diabetes [4].

LANCL (Lanthionine synthetase c-like protein) family is considered as a receptor to ABA especially $LANCL_2$ [5]. $LANCL_2$ is a protein broadly expressed in plasma and nuclear membranes of immune, epithelial and muscle cells and is considered as a potential therapeutic target for chronic inflammatory, metabolic and immune-mediated diseases [6].

The pathogenesis of gastrointestinal abnormalities in diabetes is not well understood. It may be multifactorial complex in nature involving autonomic neuropathy, motor dysfunction, glycemic control and psychological factors**.** In diabetic patients with gastrointestinal symptoms, 68% were found to have delayed gastric emptying [7].

Moreover, some researches tried to study the preventive and therapeutic effects of ABA on diabetes and inflammatory bowel disease (IBD) [(8&9)] and tried to identify its underlying mechanism of action. Hong et al. (2013) [(10)] and Sheth and Devang (2012) [(11)] found that ABA analogue SD217595 caused inhibition of K^{+} induced phasic and tonic contractions of rat bladder detrusor and both prostatic and epididymal vas deferens smooth muscle strips, While, Minorsky and Peter (2002) [12] demonstrated that ABA enhanced field stimulation responses by 25% of smooth muscle from the bladder and the vas deferens. Also, Li et al. (2011) [13] reported that ABA caused enhancement of K^{+} contracture tension by up to 400% in ileal smooth muscles. So, the physiological effects of ABA on smooth muscle contractility are not yet established.

So, the aim of our work is to: (i) study the effect of ABA on gastric smooth muscle contractility both in normal and diabetic rats (ii) try to identify the possible underlying mechanisms of action of ABA.

2. Materials and methods

2.1.Experimental Animals

Forty eight adult male Sprague-Dawley rats weighing 200-250 grams were included in this work. Animals were purchased from the Medical Experimental Research Center (MERC) of Mansoura University, Egypt. All rats are kept at room temperature of 25±2°C, with relative humidity ranged between 55% to 60% and under a 12 hours light / dark cycle. Standard pellet chow and water was daily provided. The study was approved by the research ethics committee, faculty of medicine, Mansoura University, Egypt. (Approval number: MS/17.07.47).

2.2. Experimental groups

Rats were randomly assigned to one of two groups (n = 24/group): group I: (Control group): which was subdivided into 6 subgroups, 4 rats in each subgroup; subgroup Ia: to determine the effect of ABA in different doses on gastric motility; subgroup Ib: to determine the effect of ABA on gastric motility after adding autonomic drugs (Atropine, propranolol and phentolamine); subgroup Ic: to determine the effect of ABA on gastric motility after adding Ca^{+2} channel blockers (Verapamil and propofol); subgroup Id: to determine the effect of ABA on gastric motility after adding L-NAME; subgroup Ie: to determine the effect of ABA on gastric motility after adding Na^{+} channel blocker (Lidocaine); subgroup If: to determine the effect of ABA on gastric motility after adding peroxisome proliferator - activated receptor gamma (PPAR -γ) antagonist. Group II (Diabetic group): which was subdivided into 6 subgroups, 4 rats in each subgroup; subgroup IIa: to determine the effect of ABA in different doses on gastric motility; subgroup IIb: to determine the effect of ABA on gastric motility after adding autonomic drugs (Atropine, propranolol and phentolamine) ; subgroup IIc: to determine the effect of ABA on gastric motility after adding Ca^{+2} channel blockers (Verapamil and propofol); subgroup IId: to determine the effect of ABA on gastric motility after adding L-NAME; subgroup IIe: to determine the effect of abscisic acid on gastric motility after adding Na^{+} channel blocker (Lidocaine); subgroup IIf: to determine the effect of ABA on gastric motility after

adding peroxisome proliferator - activated receptor gamma (PPAR -γ) antagonist.

2.3. Induction of type I diabetes

Diabetes was induced by intraperitoneal injection of 50 mg/kg as a single dose of freshly prepared streptozotocin, (Sigma Aldrich, SO130- 1G). 72-hours after STZ-injection, the blood glucose level was estimated [14]. Diabetic animals were kept without treatment for 8 weeks to reveal complications of diabetes [15].

2.4. Markers for diabetes

Blood glucose level was measured using commercial kit, it was measured according to the modified method illustrated by Barham and Trinder (1972) [(16)] after enzymatic oxidation by glucose oxidase. Moreover, serum insulin was measured by insulin kits for rat (ELISA) obtained from company of Sun-Red biology and technology, Shanghai #cat no 201-11-0708.

2.5. Tissue preparation

Overnight fasted rats (with free access to water) were anaesthetized by thiopental. Midline incision in the anterior abdominal wall was done then the stomach was dissected. Gastric specimens were suspended in 30 mL organ bath containing gassed Krebs solution. Krebs solution, maintained at 37°C and allowed for equilibration time [17]. At least 60 minutes were given to the preparations in the organ chamber before recording for attaining equilibrium [17]. Gastric strips (3×20mm) were prepared and kept in Krebs solution of the following composition in mM concentrations: NaCl 118.5, $NaHCO_3$ 25, KCl 4.8, $MgSO_4$1.2, KH_2PO_4 1.2, $CaCl_2$ 1.9 and glucose 10.1 (pH 7.4) [18].

2.6. Recording of gastric motility

Gastric contractility was recorded by using a Power Lab recording unit (4/30) with its isotonic transducer (Model: TRO015) that was connected to bridge amplifier. Analysis of recorded data was performed via Lab chart 7 software.

2.7. Equipments

The organ bath consists of a central tube that has a 25 ml capacity. It has an outlet at the lower end for Krebs solution drainage during wash out. A water container surrounded the tube and its temperature was always 37°C through an electrical thermostatic heater. A hollow-hooked stainless-steel tube was placed into the central tube with its main head directed downwards to supply the solution with gas and fix the specimens simultaneously.

2.8. Tissue fixation and basal recording

The selected gastric specimens were fixed vertically from both ends through clamping their lower end at the depth of the organ bath while their upper end was connected to a pin which was connected to the isotonic transducer of the Power Lab recording unit. Time of recording is 5 minutes for both basal contractility and effect of different drugs.

2.9. Chemicals

Abscisic acid: Product number A1049 obtained from SIGMA-ALDRISH; storage temperature: -20 °C. Freshly prepared by dissolving in dimethyl sulfoxide (DMSO) (20 mg/ml.). Streptozotocin: obtained from SIGMA-ALDRISH. Atropine: available from Memphis-Egypt; in the form of atropine sulphate ampoule (1mg/ml.) in a concentration of 10^{-6} M [19]. Propranolol: available from ALEX.CO. in the form of masyestrotense ampoule (1mg/ml.) ; in a concentration of 10^{-6} M [20]. Phentolamine: available from ALEX.CO; in the form of rogitamine ampoule (10 mg/ml.); in a concentration of 10^{-6}M [21]. Verapamil: available from Abbott company; in the form of isoptin ampoule (2.5 mg/ml.); in a concentration of 10^{-6} M [22]. Propofol: available from AstraZeneca; in the form of diprivan ampoule (10 mg/ml.); in a concentration of 50 micromole[23]. N^G – nitro – L – arginine methyl ester (L-NAME): available from SIGMA-ALDRISH; in the form of L-NAME powder (5 gm); in a concentration of 10^{-4} M [24]. Lidocaine: available from PHARCO; in the form of lidocaine hydrochloride ampoule (10 mg/ml.); in a concentration of 10^{-4} M [25]. Diclofenac sodium: (PPAR -γ antagonist): available from PHARCO; in the form of declophen ampoule (75 mg/ml.); in a concentration of 10^{-5} M [26].

2.10. Real PCR technique for measurement of LANCL2 expression

The kit used for RNA extraction was RNeasy mini kit purchased from QIAGEN Sample and Assay Technologies in Germany(CAT. NOS. 74104 AND 74106). While the kit used for cDNA Synthesis was SensiFAST™ purchased from BIOLINE Company (U.K), with catalog numbers: BIO-65053 and store at –20°. While the kits for real time PCR were SensiFAST™ SYBR® No-ROX Kit purchased from A BIOLINE company (U.K), with catalogue NO : BIO-98002: 200 x 20 µL reactions: 2 x 1 Ml and store at –20°C. The device used was Thermo Fisher scientific (Vantaa, Finland) SN: PR0961201124. Primers were obtained from Vivantis Technologies company, NM- 001014187.1 Rattus norvegicus LanC like 2 (LANCL2), mRNA. Forward primer GGTTGGACAGGCATAGCACT and reverse primer GTGACTCTGCGTCCACTCAA. NM- 017008.4 Rattus norvegicus glyceraldehyde-3-phosphate dehydrogenase (GAPDH), mRNA with forward primer TTGTGCAGTGCCAGCCTCGT and reverse primer TGCCGTTGAACTTGCCGTGG.

2.11. Statistical analysis

The recorded figures were analyzed by Lab Chart Reader 8 into data. Data analysis was done by the statistical package for social science (SPSS), version 22. Data comparison was done through Analysis of variance (ANOVA) with post hoc Turkey test. The data were expressed as Mean ± SD.

3.RESULTS

3.1. Effects of abscisic acid in different doses from (10^{-10} M to 10^{-4}M) on gastric contractility in control and diabetic rats

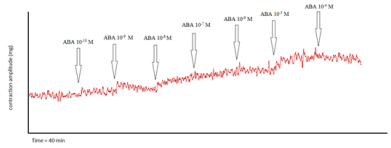
In figure (1 A) and table (1), ABA in different doses resulted in a significant increase of the gastric contractility tone of control group. 10^{-4} M ABA caused a non-significant change in the tone of control group when compared with 10^{-5}M ABA. So, the maximal dose of ABA in control group is 10^{-5}M. As regards amplitude, ABA in different doses led to a significant increase of the gastric contractility amplitude of control group. 10^{-4} M ABA caused a non-significant change in the amplitude of control group when compared with 10^{-5} M ABA. So, the maximal dose of ABA in control group is 10^{-5}M. In figure (1 B) and table (1), ABA in different doses significantly increased the gastric contractility tone of diabetic group. 10^{-4} M and 10^{-5} M ABA caused a non-significant change in the tone of diabetic group when compared with 10^{-6}M ABA. So, the maximal dose of ABA in diabetic group is 10^{-6} M ABA. Also, ABA in different doses caused a significant increase of the gastric contractility amplitude of diabetic group. 10^{-4}M and 10^{-5}M ABA caused a non-significant change in the amplitude when compared with 10^{-6}M ABA. So, the maximal dose of ABA in diabetic group is 10^{-6}

Table (1): The effect of abscisic acid in different doses from (10-10 M to 10-4 M) on gastric contractility in control and diabetic rats as regards tone (mg) and amplitude (mm)

Tone N= 10	Basal	ABA 10^{-10}	ABA 10^{-9}	ABA 10^{-8}	ABA 10^{-7}	ABA 10^{-6}	ABA 10^{-5}	ABA 10^{-4}	P[d]
Control Mean ±SD	0	0.0023[a]	0.0037[ab]	0.0052[abc]	0.0066[abcd]	0.0080[abcde]	0.0097[abcdef]	0.0098[abcdef]	<0.001*
	0	0.0001	0.0002	0.0007	0.0010	0.0012	0.0013	0.0013	
DM Mean ±SD	0	0.0509[a]	0.0919[ab]	0.1444[abc]	0.2097[abcd]	0.2627[abcde]	0.2647[abcde]	0.2667[abcde]	<0.001*
	0	0.0087	0.0131	0.0206	0.0300	0.0375	0.0378	0.0381	
P[g]	-	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	
Amplitude N= 10	Basal	ABA 10^{-10}	ABA 10^{-9}	ABA 10^{-8}	ABA 10^{-7}	ABA 10^{-6}	ABA 10^{-5}	ABA 10^{-4}	P[d]
Control Mean ±SD	0.0059	0.0074	0.0095[a]	0.0129[abc]	0.0173[abcd]	0.0203[abcde]	0.0241[abcdef]	0.0241[abcdef]	<0.001*
	0.0008	0.0011	0.0014	0.0018	0.0025	0.0011	0.0033	0.0033	
DM Mean ±SD	0.0241	0.0510	0.0654[a]	0.0937[abc]	0.1305[abcd]	0.1723[abcde]	0.1763[abcde]	0.1783[abcde]	<0.001*
	0.0049	0.0059	0.0093	0.0134	0.0186	0.0246	0.0252	0.0255	
P[g]	0.01*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	

N: number of contraction Data expressed as mean±SD
SD: standard deviation P:Probability *:significance <0.05
Pd: significance with adding multiple doses (test used: ANOVA followed by post-hoc tukey)
a:significance vs basal contraction
b: values in comparison with ABA 10^{-10} M
c: values in comparison with ABA 10^{-9} M
d: values in comparison with ABA 10^{-8}M
e: values in comparison with ABA 10^{-7} M
f: values in comparison with ABA 10^{-6} M
g: values in comparison with ABA 10^{-5} M
Pg: significance between Control & DM groups (test used: Student's t-test

A



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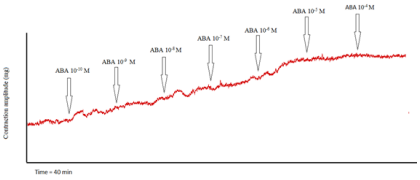


Figure (1): effect of abscisic acid in different doses from (10^{-10} M to 10^{-4}M) on gastric contractility A) in control rats B) in diabetic rats

2.1.Effects of abscisic acid (10^{-7} M) on gastric contractility in control rats

As illustrated in figure (2 A) and tables (2), abscisic acid after adding atropine caused a significant increase in the tone of control gastric contraction as compared with contractions after atropine. As regards amplitude, ABA caused a significant increase in the amplitude of same group when compared with contractions after atropine. In figure (2 B) and table (2), abscisic acid after adding propranolol caused a significant increase in the tone of gastric contraction when compared with contractions after propranolol. Also, it caused a more significant increase in the amplitude of gastric contractions when compared with contractions after propranolol. In figure (2 C) and table (2), ABA after adding phentolamine caused a significant increase in the tone of gastric contraction when compared with contractions after phentolamine. ABA, also, produced a significant increase in the amplitude of gastric contraction when compared with contractions after phentolamine. In figure (2 D) and table (2), ABA after adding verapamil caused a non-significant change in gastric contractions as regards tone and amplitude when compared with contractions after verapamil. In figure (2E) and table (2), after adding propofol, ABA caused a non-significant decrease in the tone and amplitude of gastric contraction. In figure (2 F), and table (2), ABA after adding L-NAME caused a significant increase in the tone and amplitude of gastric contractions. In figure (2 G) and table (2), ABA after adding lidocaine caused a significant increase in the tone and amplitude of gastric contractions. In figure (2 H) and table (2), ABA after adding PPARγ antagonist caused a non-significant change in the tone and amplitude.

Table (2): The effects of abscisic acid (10-7 M) on gastric contractility in control and diabetic rats as regards tone (mg) and amplitude (mm)

N= 10	Control		Diabetic		P2	P3
	Tone (mg)	Amplitude (mm)	Tone (mg)	Amplitude (mm)		
Basal con. Atropine ABA After P1	-0.0970±0.0139 -0.1159±0.0166 a -0.0710±0.0143 ab <0.001*	0.0156±0.0018 0.0115±0.0022 a 0.0167±0.0024 b <0.001*	0.0022±0.0003 -0.0194±0.0028 a -0.0112±0.0016 ab <0.001*	0.0250±0.0036 0.0184±0.0026 a 0.0315±0.0039 ab <0.001*	<0.001* <0.001* <0.001*	<0.001* <0.001* <0.001*
Basal con. Propranolol ABA After P1	0.0056±0.0008 0.0049±0.0007 0.0070±0.0014 ab <0.001*	0.0043±0.0006 0.0055±0.0008 a 0.0065±0.0008 ab <0.001*	-0.0959±0.0137 -0.1468±0.0210 a -0.0759±0.0166 ab <0.001*	0.0134±0.0019 0.0209±0.0033 a 0.0353±0.0035 ab <0.001*	<0.001* <0.001* <0.001*	<0.001* <0.001* <0.001*
Basal con. phentolamine ABA After P1	-0.0394±0.0056 -0.0374±0.0053 -0.0220±0.0032 ab <0.001*	0.0049±0.0011 0.0061±0.0019 0.0091±0.0015 ab <0.001*	-0.0580±0.0069 -0.0754±0.0122 a -0.0357±0.0080 ab <0.001*	0.0240±0.0034 0.0304±0.0036 a 0.0616±0.0045 ab <0.001*	<0.001* <0.001* <0.001*	<0.001* <0.001* <0.001*
Basal con. verapamil ABA After P1	0.0027±0.0004 -0.0268±0.0038 a -0.0282±0.0060 a <0.001*	0.0059±0.0008 0.0039±0.0007 a 0.0037±0.0004 a <0.001*	-0.0733±0.0148 -0.1254±0.0151 a -0.1232±0.0172 a <0.001*	0.0172±0.0025 0.0091±0.0013 a 0.0087±0.0005 a <0.001*	<0.001* <0.001* <0.001*	<0.001* <0.001* <0.001*
Basal con. propofol ABA After P1	-0.0007±0.0001 -0.0113±0.0016 a -0.0115±0.0028 a <0.001*	0.0144±0.0021 0.0100±0.0014 a 0.0091±0.0008 a <0.001*	-0.04918±0.00703 -0.1108±0.01583 a -0.1147±0.02067 a <0.001*	0.0240±0.0034 0.0344±0.0063 a 0.0407±0.0050 ab <0.001*	<0.001* <0.001* <0.001*	<0.001* <0.001* <0.001*
Basal con. L-NAME ABA After P1	-0.1162±0.0152 -0.0961±0.0109 a -0.0753±0.0136 ab <0.001*	0.0442±0.0063 0.0682±0.0097 a 0.0855±0.0094 ab <0.001*	-0.1069±0.0153 -0.0899±0.0128 a -0.0552±0.0122 ab <0.001*	0.0444±0.0063 0.0701±0.0113 a 0.1106±0.0087 ab <0.001*	0.18 0.25 0.0026*	0.95 0.69 <0.001*
Basal con. lidocaine ABA After P1	-0.0906±0.0129 -0.0992±0.0142 -0.0841±0.0120 b <0.001*	0.0467±0.0067 0.0334±0.0048 a 0.0598±0.0085 ab 0.045*	-0.0291±0.0042 -0.0575±0.0082 a -0.0213±0.0030 ab <0.001*	0.0243±0.0023 0.0104±0.0035 a 0.0898±0.0128 ab <0.001*	<0.001* <0.001* <0.001*	<0.001* <0.001* <0.001*
Basal con. PPARγ antag. ABA After P1	-0.0613±0.0088 -0.0825±0.0118 a -0.0847±0.0128 a <0.001*	0.0175±0.0025 0.0148±0.0021 a 0.0144±0.0022 a 0.01*	-0.07524±0.01075 -0.09413±0.01402 a -0.0982±0.01714 a 0.0027*	0.0273±0.0038 0.0208±0.0033 a 0.0194±0.0037 a <0.001*	0.005* 0.06 0.06	<0.001* <0.001* 0.0018*

Data expressed as mean±SD N: number of contraction
SD: standard deviation P:Probability *:significance <0.05
P1: significance within either control or DM group(test used: ANOVA followed by post-hoc tukey)
a:significance vs basal contraction
b: significance vs blocker
P2: significance between Control & DM groups as regard tone(test used: Student's t-test)
P3: significance between Control & DM groups as regard amplitude(test used: Student's t-test)

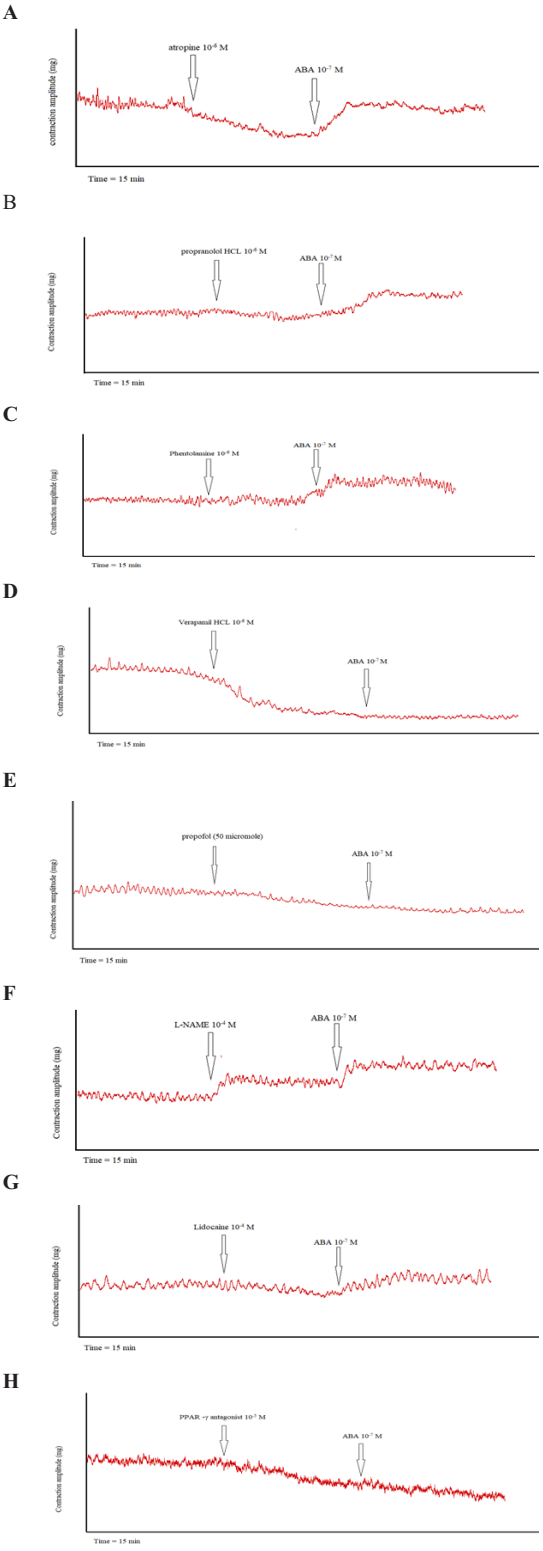
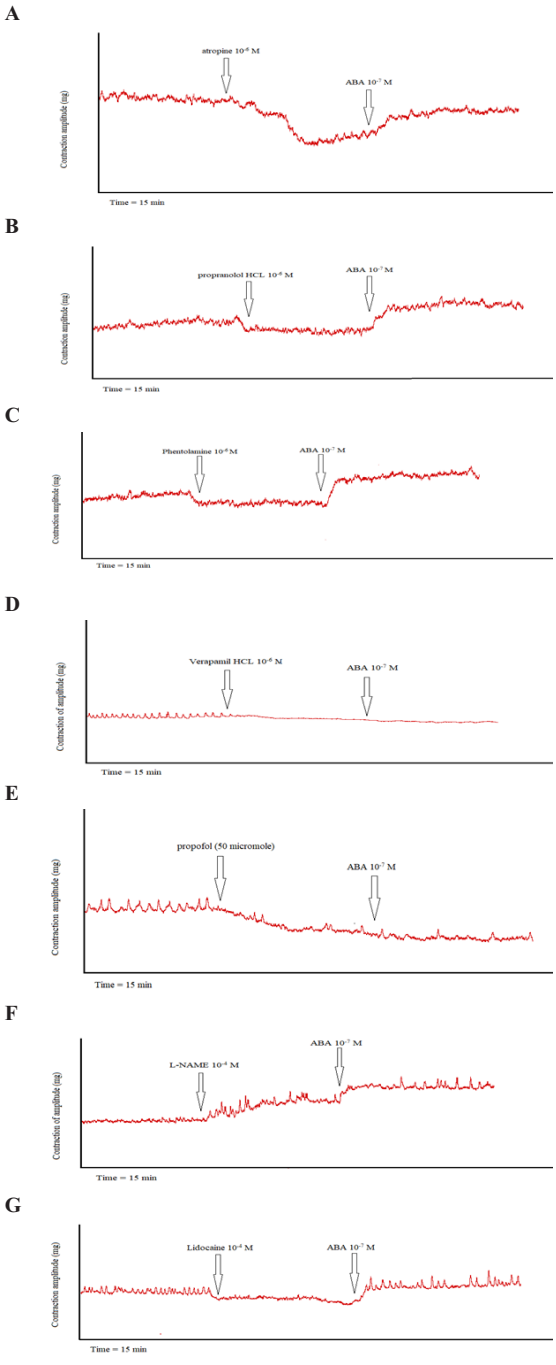


Figure (2): effect of abscisic acid (10^{-7} M) on gastric contractility in control rats. A) after adding atropine (10^{-6} M) B) after adding propranolol hydrochloride (10^{-6} M) C) after adding phentolamine (10^{-6} M) D) after adding verapamil hydrochloride (10^{-6} M) E) after adding propofol (50 micromole) F) after adding L-NAME (10^{-4} M) G) after adding Lidocaine (10^{-4} M) H) after adding PPARγ antagonist (10^{-5} M)

2.1. Effects of abscisic acid (10^{-7} M) on gastric contractility in diabetic rats

In figure (3 A) and table (2), Abscisic acid after adding atropine caused a significant increase in the tone of gastric contraction when compared with contractions after atropine. Also, it caused a significant increase in the amplitude of gastric contraction when compared with contractions after atropine. In figure (3 B), and table (2), Abscisic acid after adding propranolol caused a significant increase in the tone of gastric contraction when compared with contractions after propranolol. ABA, also, caused a significant increase in the amplitude of gastric contraction when compared with contractions after propranolol. In figure (3 C) and tables (2), Abscisic acid after adding phentolamine caused a significant increase in tone when compared with contractions after phentolamine. Also, it caused a significant increase in amplitude of gastric contractions when compared with contractions after phentolamine. In figure (3 D) and table (2), ABA caused a non-significant change in the tone and amplitude of gastric contractions. In figure (3 E) and table (2), ABA after adding propofol caused a non-significant decrease in the tone of gastric contraction but caused a significant elevation of the amplitude. In figure (3 F) and table (2), ABA after adding L-NAME caused a significant increase in the tone and amplitude of gastric contractions. In figure (3 G) and table (2), ABA after adding lidocaine caused a significant increase in the tone and amplitude of gastric contraction. In figure (3 H) and table (2), Abscisic acid after adding PPARγ antagonist caused a non-significant change in tone and amplitude of gastric contractions.



H

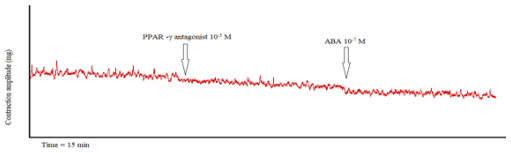


Figure (3): effect of abscisic acid (10^{-7} M) on gastric contractility in diabetic rats. A) after adding atropine (10^{-6} M) B) after adding propranolol hydrochloride (10^{-6} M)C) after adding phentolamine (10^{-6} M) D) after adding verapamil hydrochloride (10^{-6} M) E) after adding propofol (50 micromole) F) after adding L-NAME (10^{-4} M) G) after adding Lidocaine (10^{-4} M) H) after adding PPARγ antagonist (10^{-5} M)

3.4. Expression of mRNA of LANCL2 receptor in gastric smooth muscle cell in control and diabetic groups.

The results of expression of mRNA of LANCL2 gene are presented in table (3). The table shows a significant increase in relative expression of LANCL2 mRNA in diabetic group ($P < 0.05$) compared to control group.

Table (3): mRNA expression of LANCL2 receptors in gastric smooth muscle in control and diabetic groups.

Gene expression	Mean± SD	P value
Control stomach	0.72 ± 0.1	0.007
Diabetic stomach	0.99 ± 0.2	

Data expressed as mean±SD SD: standard deviation
P:Probability *:significance <0.05

4. DISCUSSION

Diabetes related gastrointestinal manifestations are not only prevalent but rather disturbing and incapacitating. The pathophysiology of diabetic gastroenteropathy is the cumulative result of multiple factors. In addition to autonomic neuropathy [27], the role of enteric nervous system is becoming more evident [28] besides ICC affection [29]. Nonetheless, DM-related gastric motility disorders can be the outcome of changes of other elements such as: oxidative stress, growth factors and diabetes provoked changes in gastrointestinal smooth muscles.

ABA has been identified as a new human signaling molecule that adjusts metabolic, nutritional, inflammatory, and immunological responses and it has a hand in glycemic control through a LANCL2 dependent mechanism [30]. The effect of ABA regarding diabetes has been confirmed in rats in the form of blood glucose level regulation and preventing diabetic onset [31].

The effect of ABA on contractility of smooth muscles is debatable. Huddart et al. (1986) [32] issued that 10^{-6} M ABA displayed about 25% field stimulation responses enhancement on smooth muscles of rat vas deferens and bladder. Moreover, Samir and Mostafa (2018) [33] proved that ABA has provocative effects on myometrial contraction in normal and diabetic rats. In contrast, Masters et al. (1994) [34] detected that ABA analogue SD217595 has a substantial capacity for blocking Ca^{+2} entry in rat smooth muscle preparation of prostate and vas deferens, also, Lynch (1991) [35] demonstrated that ABA analogue SD217595 inhibits K^+ induced phasic and tonic contractions of rat bladder. While Sheth (2012) [(11)] reported that Abscisic acid of 1, 10 and 100 µg/ml. concentrations exerted no effect on isolated ileum of rat.

In this study, ABA exhibited a stimulatory effect on basal gastric contractility in both normal and diabetic rats with significant increase in the diabetic group compared to control. On exposing the normal gastric strips to different ABA concentrations (10^{-10} M to 10^{-4} M), our findings displayed a significant rise in the tone and amplitude of contractions with increasing ABA concentration reaching E max at 10^{-5} M. While in diabetic group, ABA at different concentrations (10^{-10} M to 10^{-4} M) displayed a more significant rise in gastric contractility regarding the tone and amplitude with E max at 10^{-6} M. These results are in agreement with Samir and Mostafa (2018) [33] who reported that ABA has provocative effects on myometrial contraction in both normal and diabetic rats.

Moreover, this study revealed a significant increase in LANCL2 receptors mRNA expression in gastric tissue in diabetic group. LANCL2 has been identified as a critical component of the ABA-sensing protein complex [36]. In addition, LANCL2 blocking abolished the rise of cytoplasmic Ca^{2+} ($Cacyt^{2+}$) and cAMP elicited by ABA [31].

In the present work, diabetes significantly decreased the tone and amplitude of gastric contractility compared to control. These results are consistent with James et al. (2004) [37] who found that fundal contractions decreased in diabetic mice compared to controls and suggested that these changes may have important role in diabetic gastric dysmotility. Also, Altan et al. (1987) [(38)] reported a reduced gastric contractility in diabetic rats that was related to reduced beta adrenergic and/or serotonergic receptors activity and not related to impaired muscarinic receptors or adenyl cyclase activity. Min et al. (2018) [39] attributed that gastric dysmotility was due to impaired inhibitory nitrergic and excitatory cholinergic neuronal pathways in the stomach of diabetic patients. While Mahavadi et al. (2017) [(40)] reported that fundal contractility obtained from mice stomach increased when exposed to hyperglycemic medium and confirmed that oxidative stress in DM can increase smooth muscle contractility.

Muscrinic receptors interact with G protein to activate phospholipase c which increases IP_3 and DAG thus increasing Ca^{2+} intracellular. This activates MLCK which phosphorylate myosin to interact with actin to cause smooth muscle contraction [41]. In this study, blocking of these receptors doesn't abolish excitatory effects of ABA on gastric motility indicating that these receptors are not probably involved in the excitatory effects of ABA.

β adrenergic receptors, via G protein, decrease cytosolic calcium and the sensitivity of contractile proteins to calcium and leads to relaxation of the wall of smooth muscles [42]. Also, presynaptic α2 receptors decrease the release of excitatory neurotransmitters or decrease release of inhibitory neurotransmitters [43]. In the present study, β and α adrenergic receptors were blocked by propranolol and phentolamine respectively. ABA addition after propranolol caused a significant increase in the tone of gastric contractions. ABA addition after phentolamine caused a significant increase in the tone of gastric contraclions. These observations mean that ABA effects on the gastric motility are not mediated via adrenergic receptors mechanism.

Ca^{2+} entry through L type Ca^{2+} channels is the primary mechanism for excitation contraction coupling in gut smooth muscles [44]. In the present study, the excitatory effect of ABA is diminished after blocking of both Ca^{2+} channels (L type) by verapamil and (T type) by propofol. These results confirm the involvement of Ca^{2+} in mechanism of action of ABA which agrees with Huddart et al. (1986) [32] who published similar results on vas deferens and bladder of rats. Also, in agreement with Samir and Mostafa (2018) [(33)] who reported a decrease in the excitatory effect of ABA after blocking L-type calcium channels in the smooth muscle of uterus.

NO relaxes smooth muscles by direct action and indirectly by inhibiting release of the excitatory neurotransmitters acetylcholine and substance P [45&46]. The addition of the nitric oxide synthase inhibitor, L-NAME before application of ABA caused a significant increase in the amplitude and the tone of gastric contractions. Our results demonstrated that, ABA still has an excitatory effects on gastric motility after blocking of NO synthesis by L-NAME. These observation means that the stimulatory effects of ABA are not mediated through NO inhibiting synthesis and release mechanism.

In the present work, lidocaine significantly decreased gastric contractions. This inhibitory effect of Na^+ channel blockers could result from either membrane stabilizing effect by blocking Na^+ channels or from inhibition of opening of voltage gated Ca^{2+} channels [47]. The observed excitatory effects of ABA that still occur after adding lidocaine suggesting that ABA effect on gastric motility may not involve Na^+ channels mechanism.

Activation of PPARγ in human myometrium via a thiazolidinedione elicits a rapid increase in Ca^{2+} in myocytes [48]. In this study, PPARγ receptor antagonist had inhibitory effect on gastric motility in both groups which in turn abolished stimulatory effects of ABA. So, PPARγ receptor may be involved in the mechanism of action of ABA.

Bassaganya-Riera et al. (2010) [49] observed that ABA is not a direct ligand of PPAR-γ's ligand-binding domain and it is believed to activate the PPARγ pathway indirectly by binding to upstream proteins such as LANCL2 and triggering a signaling cascade. LANCL2 has been identified as a critical component of the ABA-sensing protein complex

[37]. Moreover, LANCL2 knockout studies demonstrated that ABA-mediated activation of macrophage PPARγ was dependent on LANCL2 expression [49] suggesting a potential cross-talk between PPARγ and LANCL2. Furthermore, ABA treatment increased cAMP accumulation in immune cells [49] indicating that additional mechanisms of action are implicated in the anti-inflammatory effects of ABA. Surprisingly, the loss of PPARγ in intestinal epithelial cells resulted in upregulation of LANCL2 in epithelial cells, suggesting that PPARγ may play an inhibitory role in the transcriptional regulation of LANCL2 through a feedback loop [50].

ABA activates PPARγ in pre-adipocytes [51], and a deficiency of PPARγ in immune cells impairs the ability of ABA to normalize blood glucose concentrations and ameliorate macrophage infiltration in the white adipose tissue of obese mice [8]. The ABA mediated activation of PPARγ can be blocked by inhibiting intracellular cAMP production or protein kinase A (PKA) activity [52], suggesting that ABA triggers an alternative mechanism of PPARγ activation. However, no data are available addressing whether ABA activates PPARγ by binding to its ligand-binding domain or through an alternative mechanism involving indirect interactions with PPARγ through LANCL2 receptors.

5. CONCLUSIONS

ABA has excitatory effect on basal gastric motility with higher response in diabetic stomach and this response is in correlation with real time PCR results which showed over expression of mRNA of LANCL2 receptors in diabetic tissue. Also, ABA has excitatory effect on gastric contractility in both control and diabetic groups after blocking of autonomic receptors. This result confirms that its effect is not mediated via these receptors. Moreover, Ca^{2+} channel blockers diminished the excitatory effect of ABA on gastric tissue in control and in diabetic groups. So, the excitatory effect of ABA involves Ca^{2+} channels in its mechanism of action. Also, ABA caused a significant excitatory effect after adding L-NAME in both groups in gastric motility. In addition, ABA has stimulatory effect on gastric motility in both groups after adding lidocaine. So, Na^{+} channels are not implicated in ABA effects on gastric segments. In addition, Excitatory effect of ABA can be abolished after adding PPAR –γ antagonist and this observation confirm the relation between ABA, LANCL2 receptor and PPAR –γ receptor.

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