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Research Article

Evaluation of the effect of metformin on the inhibitory effect of oxytocin on potassium chloride stimulated goat ileum

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ABSTRACT

The posterior pituitary hormone, oxytocin is expressed in the myenteric and submucous ganglia and nerve fibers along the entire human gastrointestinal (GI) tract. The role for oxytocin in the physiology and pathophysiology of the bowel remains to be clearly elucidated. Many studies have described that oxytocin exerts stimulatory or inhibitory effects on gut functions. Recently, mRNA for oxytocin and its receptor was found throughout the entire human GI tract. In this study, we examined the responses of the posterior pituitary hormone, oxytocin on the contractile responses to KCl and the effect of metformin on these responses as it affects the glucose transport and causes monoamine release in the gut.

Keywords: Oxytocin, Ileum, Glucose, Contractile, Potassium, Gut

INTRODUCTION

Oxytocin is a hormone with its most well-known effects on myoepithelial cells and uterine smooth muscles in the responses associated with the milk ejection reflex and parturition. During the last few years, the possibility has been raised that oxytocin also contributes to the control of the gastrointestinal (GI) motility. Oxytocin is secreted into the blood endogenously in response to after a fatty meal, and cholecystokinin (CCK) release in women.[1,2] Intravenous infusion of oxytocin leads to increased colonic peristalsis, and to accelerated gastric emptying of a meal in healthy subjects,[3] whereas an oxytocin receptor antagonist delays the gastric emptying.[4] In contrast, gastric motility is inhibited in dogs and rats.[5] Oxytocin relaxes the ileum and caecum, while it has a contracting effect on colon, both *in vitro* and *in vivo*.

Although there are divergent results in different species, the effects observed throughout the GI tract suggest that there may be receptors for oxytocin in the gut. It has recently been shown that mRNA for oxytocin and its receptor are expressed throughout the human GI tract.[1] On the other hand, metformin (1.1 dimethylbiguanide hydrochloride)belongs to the biguanide class of anti-diabetic drugs that are guanidine derivatives. More recent studies have shown that metformin delays the intestinal absorption of glucose, which occurs more distally, and stimulates utilization of intestinal glucose, particularly anaerobic metabolism, which contributes to a reduction of glucose uptake in both animals and humans.[6]

METHODS

Drugs and chemicals

Metformin (Franco Indian Pharmaceuticals, India), oxytocin (Novartis Pharmaceuticals, India), potassium chloride (Fisher Scientific, India) and all other reagents and chemicals obtained were of standard quality and obtained from locals chemical stores.

Isolated goat ileum preparation

The technique used was a modified version. A goat ileum was procured from local sources. A piece of goat ileum was removed, cleaned and was placed in a petri dish containing Tyrode solution. A thread was attached to the top to serve as a marker. The perfusion fluid in petri dish was aerated and debris inside the lumen was washed gently with pipette. The mesenteric membrane was trimmed for a length of ileum of approximately 2 cm. Two threads were tied to the upper and lower portion of the gut. The thread tied to the lower portion was attached to the hook of the air-delivery tube inside the bottom of the chamber, in a water-jacketed organ bath containing 20 ml Tyrode solution (composition in mM: NaCl 136.89, KCl 2.68, $MgCl_2$ 1.05. $CaCl_2$ 1.36, NaH_2PO_4 0.32, $NaHCO_3$ 11.90 and glucose 5.55) and the thread tied to the upper portion of gut was attached to the force displacement transducer. Tissues were mounted under an initial load of 1.0 g and allowed to equilibrate for 30 min. before the addition of any drug. The experiments were performed at 37°C and bubbled with a mixture of air produced by a motorized areator. Normal rhythmic motility was recorded on a student's electric kymograph (Bio-Device, Ambala). The effect of KCl (60 mM) with and without pre-treatment with Oxytocin (6 µg) was tested on spontaneous contractions of goat ileum segment. Each concentration tested was allowed a contact time of 1 min followed by washing three times with the Tyrode solution. A resting period of 15 min was allowed before the next addition. In a separate set of experiments, metformin was added 25 min prior to oxytocin treatment and the effect on the ileal smooth muscle contractility ascertained.

Glucose transport in ileum

In another set of experiments (n=5), about 5 cm long goat ileum was mounted in a student organ bath containing ringer solution. Tissue were maintained at 32°C ± 1°C, aerated with air and resting load of 1 g. 1% glucose solution was prepared and inserted in the ileum of goat using syringe.[7] The tissue was mounted in the inner organ bath (receiver environment) containing Ringer's solution. The glucose concentration which transported to the inner organ bath was estimated by SAPS protocol.[8]

RESULTS

The results obtained from the experiments are described in Table 1. These results were statistically evaluated using the student's t test. KCl when used in (60 mM) produced contraction indicative of an interaction with cells of the smooth muscle in the ileum. On the other hand, pre-treatment with the Oxytocin 6 µg reduced the KCl induced contractions by 22.33 % ($p<0.05$). However, pre-treatment with Metformin restored this effect to some extent and the relaxation induced was only 8.62 % ($p>0.05$). The effect of metformin was evaluated on the glucose transport; however in our studies, it did not show any significant alteration and caused a reduction of only 7.9% in glucose levels (data not shown).

DISCUSSION

Oxytocin is a hormone best known for its effect on myoepithelial cells and uterine smooth muscle in responses associated with the milk ejection reflex and parturition. During recent years, increasing evidence has indicated that oxytocin plays a role in the regulation of functions of the GI tract. Previous studies have suggested that the effects of oxytocin on GI motility are species dependent and vary in responses when applied to human, guinea pig, rat etc. For example, oxytocin accelerates[9,10] and oxytocin receptor antagonists delay gastric emptying in humans,[1] but oxytocin delays gastric emptying in rats.[5] The motility in the stomach, ileum and caecum was inhibited, while the motility in the colon was stimulated, in several animal species.

Recently reported localization data for oxytocin receptors show that oxytocin receptors are expressed widely in the digestive tract, such as the intestinal absorption epithelium and gland epithelium, the smooth muscles, and the enteric neurons, especially the myenteric plexus.[11]

In our study, we found relaxation of the KCl contracted ileum by about 22.3 % after pre-treatment with oxytocin. While the anti-diabetic drug metformin caused a marginal reversal of this action although this was statistically insignificant. Previous studies attribute the effects of

Table 1: Effect of metformin (4 mM) and oxytocin (6 µg) on KCl (60 mM) induced contractions in isolated goat ileum.

Pre-treatment	Treatment (60 mM)	Amplitude (height in cm)	Effect	% Change	P value
A) Nil	KCl	3.78	Contraction		
B) Oxytocin (6 µg)	KCl	3.09	Relaxation	22.3	p<0.05 A:B
C) Metformin (4 mM)	KCl	3.93	Contraction	3.96	NS A:C
D) Metformin (4 mM) + oxytocin (6 µg)	KCl	3.48	Contraction	8.62	NS B:D

Statistical comparisons shown in the last column. p>0.05 is non-significant

metformin to its inhibitory actions on glucose transport. However, although we found some reduction in glucose levels i.e., about 7.9 % it was found to be statistically insignificant. There is a possibility of oxytocin receptors being present in the ileum similar to those found in the humans, although in the rat the expression also has been recently observed. Therefore, the effects could either be receptor mediated or through modulation of neurotransmitter release. This might explain why the effects evoked by oxytocin on gastric and intestinal motility in mammalian models like the rat are suggested to be mediated by release of CCK and CCK receptors, which in turn leads to motility inhibition in the proximal GI tract. CCK is produced by endocrine cells in the proximal small intestine and is released into the blood. The question is how oxytocin mediates this release of CCK, and the oxytocin receptor antagonist atosiban inhibits the release, if no oxytocin receptors are present in the GI. In an intact animal theoretically, intraperitoneal oxytocin and atosiban injections may cross the blood-brain barrier and exert central effects by activating respective inhibit vagal neurons in the dorsal vagal complex that are involved in the regulation of CCK secretion. However, other, as yet unknown, mechanisms may be involved.

However, experimental studies have also found that metformin induces serotonin release, independent of the 5-HT3 receptor, by human duodenal mucosa through neural and non-neuronal mechanisms and whether a similar release by ileum occurs is not yet ascertained.[12]

Therefore in conclusion based on this study, we can suggest a role for oxytocin in the goat ileum; however, more comprehensive studies are required to elucidate the role of oxytocin in a mammalian intestine.

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