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ORIGINAL PAPER

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Effects of Bethanecol, Carbachol and Prostaglandin E2 on Fluid Transport across the Small Intestine of the Gerbil (*Gerbillus cheesmani*)

Abstract: The effects of bethanecol and carbachol on fluid transport across the jejunum and ileum of fed and starved (four days) gerbils (*Gerbillus cheesmani*) were investigated. The effects of prostaglandin E2 (PGE2) on fluid transport across the jejunum and ileum of fed, starved and undernourished (50% of control food intake for 21 days) were also investigated. Bethancol and carbachol had no significant effects on fluid absorption in the fed jejunum but reversed fluid absorption into secretion in the starved jejunum. Similarly, in the ileum bethanecol and carbechol caused the basal absorptive tone measured in the fed ileum to be converted to secretion and increased significantly fluid secretion in the starved ileum. Moreover, in the ileum both bethanecol-induced fluid secretion as well as carbachol-induced fluid secretion was significantly higher in the starved ileum when compared with the fed control. In the jejunum taken from fed, starved and undernourished gerbils, PGE2 reversed normal absorption into secretion and the amount of fluid secreted as a result of the presence of PGE2 in the starved and undernourished jejunum was significantly higher than in the fed jejunum. The basal fluid absorption in the fed ileum was converted to secretion in the presence of PGE2. Fluid secretion in the presence of PGE2 was significantly enhanced in the ileum taken from starved and undernourished gerbils. Thus, starvation sensitizes the jejunum and ileum to the effects of bethanecol and carbachol, while starvation and undernourishment sensitizes the two regions of the small intestine to the effects of PGE2.

Keywords: Bemthanecol, carbachol, prostaglandin E2 effect, gerbill, *Gerbillus chesmanic*, ileum, fluid transport.

تأثير مواد البيثانيكول والكاربيكول والبروستاجلاندين E2 على إمتصاص السوائل في الأمعاء ألدقيقه للجربوع جربيلاس تشيمانى (*Gerbillus cheesmani*)

فوزيه البالول

المستخلص: تمت دراسة تا ثير مواد البيثانيكول و الكاربيكول على امتصاص السوائل، خلال منطقتي الصائم واللفائفى. من الأمعاء الدقيقه والتي أخذت من جرا بيع كاملة التغذية أو ا لصائمة (لمده أربعه أيام). كما تمت دراسة تأثير مادة البروستاجلاند ين2 E على إمتصاص الشوا ئل خلال هاتين المنطقتين، فى جرا بيع كاملة التغذية وأخرى صائمة، وفى مجموعة غير كاملة التغذية (أعطيت نصف كميه الغذاء المستهلك للحيوا نا ت كاملة التغذية لمدة 21 يوم). لم تسبب مادتي البيثانيكول والكاربيكول أى تأثير على الامتصاص في منطقه الصائم التي أخذت من الحيوانات كاملة التغذية، ولكنها عكست الإمتصاص إلى إفراز في نفس المنطقة من الحيوانات الصائمة .وكذلك لم تسبب هاتين المادتين أى تأثير على امتصاص السائل في منطقه اللفائفي المأخوذه من الحيوانات كاملة التغذية، و كانت كميه السائل المفرز نتيجة لوجود هاتين المادتين في منطقه اللفائفى، أكثر في الحيوانات الصائمة أو غير كاملة التغذية مقارنة بالحيوانات كاملة التغذية. أما مادة البروستاجلاندين E2 ، فأنها عكست الامتصاص إلى إفراز في منطقه الصائم التي أخذت من المجاميع الثلاثة من الجرابيع، وكانت كميه السائل الذي تم أفرازة نتيجة لوجود هذه المادة في الحيوانات الصائمة، وغير كامله التغذية، أكثر منها في الحيوانات كامله التغذية. وكذلك عكست مادة البروستاجلاندين E2 الامتصاص إلى إفراز في منطقه اللفائفى المأخوذة من الحيوانات كامله التغذية، وكانت كميه السائل الذي تم إفرازه نتيجة لوجود هذه المادة فى الحيوانات الصائمة، أو غير كامله التغذية، أكثر منها في الحيوانات كاملة التغذية. يستنتج من هذة الدراسة إن تجويع الجرابيع (الصوم الكامل) يزيد من استعداد الأمعاء ألدقيقه للتأثر بمادتي البيثانيكول و الكاربيكول ،كما أن التجويع (الصوم الكامل أو إعطاء نصف كميه الغذاء لمدة21 يوم) يزيد من إستعداد الأمعاء الدقيقه للتأثر بمادة البروستاجلا ندين E 2.

كلمات مدخلية: البيثانيكول، الكاربيكول، البروستاجلاندين E 2 , تأثير، جربوع، جربيلاس تشيماني، أمعاء دقيقة، إمتصاص سوائل.

Introduction

There are two groups of secretagogues which cause hypersecretion in the small intestine: those that act by raising the level of intracellular calcium in the enterocyte (e.g. bethanecol and carbachol, stable cholinergic agonists) and those that act by increasing its intracellular concentration of cyclic AMP (e.g. prostaglandin E2). Mall, *et al.* (1998)

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claimed that carbachol acting via $[Ca^{2+}]i$ can induce Cl^- secretion only in the presence of cAMP, i.e., when luminal Cl^- channels are already activated. On the other hand, Toriano, *et al.* (2001) investigated water movements in T84 cells and found that water movements were not associated with a Cl^- net exit. They also showed that the basal as well as the pharmacologically induced water movements (absorptive or secretory) were coupled to different electrogenic and non-electrogenic transfers. Fasting and malnutrition enhance ion transport responses to a broad range of intestinal secretagogues which include agonists of cyclic nucleotide-dependent pathways (cAMP and cGMP), as well as those that stimulate intracellular calcium mobilization (Carey, *et al.* 1994, Young and Levin, 1990a). Prostaglandins, especially of the E series, are potent chloride secretagogues likely to be active under both physiological and pathophysiological circumstances (Weymer, *et al.* 1985). Rask-Madsen, *et al.* (1990) suggested that cholera toxin stimulates the release of 5-hydroxytryptamine, which in turn causes the release of prostaglandin E2 (PGE2).

Because of the lack of focused studies on the effect of secretagogues on fluid transport across the small intestine of desert mammals in general and on the gerbils in particular, the present study investigated the effects of bethanecol, carbachol and prostaglandins E2 on fluid transport in fed as well as in nutritionally-deprived states. That the gerbil may be able to switch intestinal secretion on and off is of importance in that it could provide new insights into the causes of terminal diarrhea in starving humans.

Materials and Methods

Animals and Diet

Gerbils of both sexes, body weight 36-40 gm, were captured from the desert and kept in the animal house for at least three weeks before use. Three nutritional groups were used. The fed group had free access to water and food (SDS Rodent maintenance diet, Essex, England). The starved group was given water *ad lib* but food was removed for 4 days before the animals were used. A chronically undernourished group was housed in individual cages and was fed 50% of the control food intake for 21 days. Animals were housed routinely in plastic cages with wire mesh bottoms to reduce coprophagy and were held in rooms maintained at 27±2°C. Lights were on from 5 am until 5 pm and the humidity was 50%.

Methods

Fluid movements were measured in anaesthetized gerbils (thiopentone sodium, 30 mg/kg body weight, i.p) *in vivo* using the gravimetric technique as described previously (Young and Levin, 1992). Approximately 0.25-0.5 ml of 0.9% NaCl was instilled into the ligated loops (6-7 cm in length) of either the jejunum or ileum from a weighed, fluid-filled syringe (Agm). The syringe was then reweighed (Bgm) and the weight of fluid instilled, sufficient to just distend the loop, was calculated by difference (A-Bgm). Fluid movement into and out of the loops was obtained for 30 min. At the end of the period the loop was excised, blotted free of excess fluid and weighed (Cgm). It was then cut open, drained, blotted and reweighed (Dgm). The amount of fluid recovered was obtained by subtraction (C-Dgm). The net movement of fluid into or from the loop was calculated as [(C-D)-(A-B)] gm. If absorption took place there was a net loss of fluid from the loop and the sign of fluid movement is given as negative but if secretion occurred there was a net gain of fluid into the loop and the sign of the fluid movement is given as positive. Animals were injected ip with bethanecol (carbamyl-*B*-methylcholine chloride 55 mg/kg body weight in 0.1 ml 0.9% NaCl) or carbachol (carbamylcholine chloride 60 mg/Kg body weight in 0.1ml 0.9% NaCl). Control experiments were undertaken by ip injection of an equal volume of 0. 9% NaCl. The experiments to assess the effects of PGE2 were conducted by instilling 0.25-0.5 ml of 0.9% NaCl containing 10mM;-11.15-Dihydroxy-9-oxoprosta-5,13-dienoic acid into the lumen of the closed loop for 30 min. Results are expressed as $(mg\ cm^{-1}\ (30\ min)^{-1})$ and are given as the mean ± S.E.M. Data from different groups were compared by a one-way Anova test, part of the SPSS program (Windows, Version 7) and were screened for differences using Student's unpaired t-test. Differences were considered significant when $P<0.05$.

Chemicals

All the chemicals were obtained from Sigma Company, St. Louis, USA.

Results

Effects of bethanecol and carbachol on fluid transport in the jejunum and ileum

The effects of bethanecol and carbachol on fluid transport in the jejunum taken from fed and starved gerbils are shown in Fig. 1A. Both drugs had no significant effects on fluid absorption in the fed jejunum. In the starved jejunum both bethanecol and carbachol reversed fluid absorption into secretion. The effects of bethanecol and carbachol on fluid transport in the ileum taken from fed and starved gerbils are shown in Fig. 1B. In the fed ileum both bethanecol and carbachol reversed fluid absorption into secretion while in the starved ileum both drugs significantly increased fluid secretion. Moreover, bethanecol-induced fluid secretion as well as carbachol-induced fluid secretion was significantly higher in the ileum from starved animals when compared with the ileum of the fed controls.

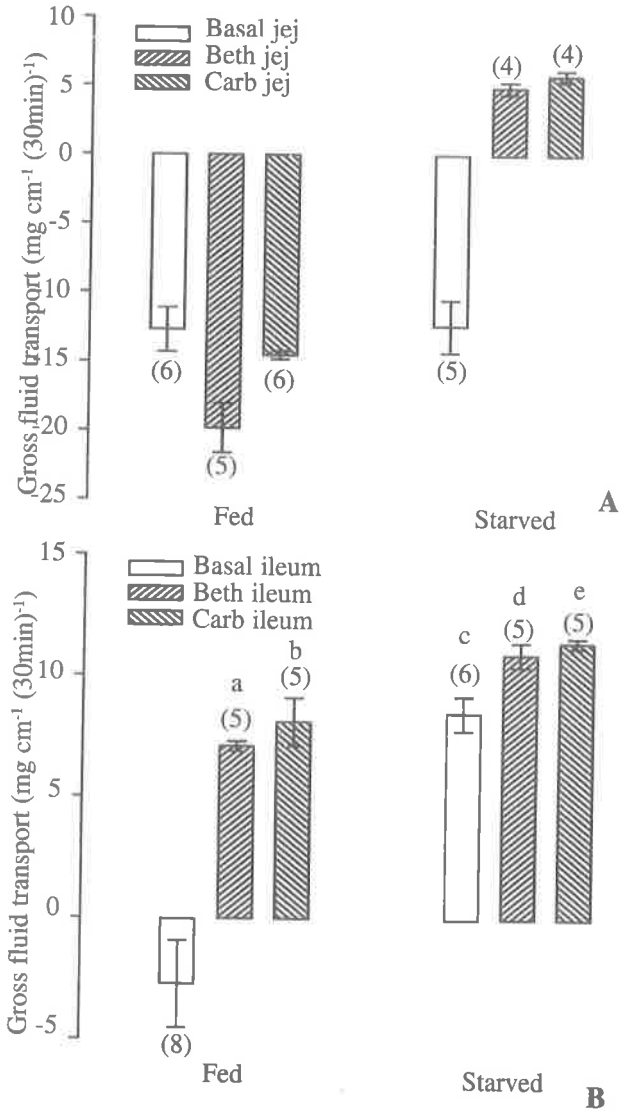

Fig.1. Effects of bethanecol and carbachol on fluid transport across the jejunum (A) and ileum (B) of fed and starved gerbils. Data are shown as mean ±SE with number of animals in brackets Secretion is +, absorption -. b *v* e; c *v* d; P<0.05. a *v* d; c *v* e; P<0.001.

Effects of PGE2 on fluid transport in the jejunum and ileum

The effects of PGE2 on fluid transport in the fed, starved and undernourished jejunum are shown in Fig. 2A. In the jejunum taken from gerbils in the three feeding conditions, PGE2 reversed normal absorption into secretion and the amount of fluid secreted as a result of the presence of PGE2 in the jejunum from starved and undernourished gerbils was significantly higher than in the fed controls. The effects of PGE2 on fluid transport in the ileum taken from fed, starved and undernourished gerbils are shown in Fig. 2B. The basal fluid absorption in the ileum from fed animals was converted to secretion in the presence of PGE2. Fluid secretion in the presence of PGE2 was significantly enhanced in the ileum taken from starved and undernourished gerbils when compared with the ileum of the fed gerbils.

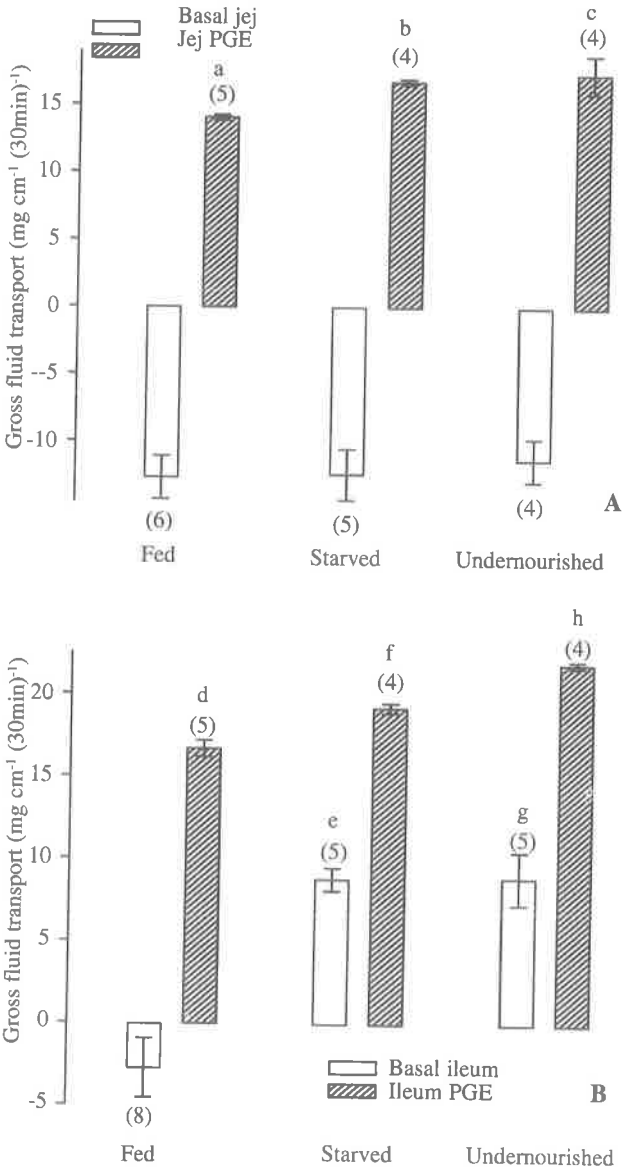

Fig. 2. Effects of PGE2 on fluid transport across the jejunum (A) and ileum (B) of fed, starved and undernourished gerbils. Data are shown as mean ±SE with number of animals in brackets Secretion is +, absorption -. a *v* b; a *v* c; d *v* f; $P<0.05$. e *v* f; g *v* h; $P<0.001$.

Discussion

Bethanecol had no significant effects on fluid absorption in the jejunum taken from fed gerbils but reversed fluid absorption into secretion in the fed ileum. This is in agreement with the results of Young and Levin (1990 a & b) in the rat small intestine. Similarly, carbachol had no significant effects on fluid absorption in the fed jejunum but it reversed fluid absorption into secretion in the fed ileum. Athman, *et al.* (2002) showed that carbachol decreased fluid absorption by ~1/3 during the initial 15 min of its administration in the mouse small intestine (using both wild-type mice and mice whose villlin gene had been invalidated). Therefore, it can be concluded that in the ileum taken from fed gerbils, there is a considerable amount of bethanecol and carbachol-induced fluid secretion.

The effects of starvation and undernourishment on the basal fluid transport in the jejunum and ileum of gerbils has been shown previously by Al-Balool (Unpublished observations 1). Neither starvation nor undernourishment had any effect on jejunal basal fluid absorption, but both conditions reversed the normal basal fluid absorption measured in the fed ileum into secretion. In the present study both bethanecol and carbachol not only caused the basal absorptive tone measured in the fed ileum to be converted to secretion, but also induced a much greater secretion in the starved ileum when compared with the fed control. Young and Levin (1990 a & b) found that in the starved jejunum and ileum (2-3 days) bethanecol as well as carbachol-stimulated fluid secretion was much greater than that of fed controls. Thus, in the gerbil as in the rat, starvation makes the two regions of the small intestine hypersensitive to the effects of bethanecol and carbachol. In the rat small intestine the enhanced secretion of fluid on day 3 of starvation was concomitant with a significant increase in the concentration of bicarbonate and chloride ions both in the basal unstimulated state and after secretion was stimulated by behanechol, carbechol and prostaglandins (Young and Levin 1990 a & b). Carey, *et al.* (1994) studied the effects of starvation on the ion transport in the piglet jejunum and found that carbachol had a very different effect on jejunal ion transport in the two nutritional states: stimulation of electrogenic Cl^- secretion in fed piglets, and inhibition of Na^+ and Cl^- absorption leading to large increases in net secretion of both ions in the fasting animals. In a previous study (Al-Balool, Unpublished observations 2) it was shown that the short circuit current (Isc) in the jejunum and ileum taken from starved and undernourished gerbils was more sensitive than in the jejunum and ileum taken from fed animals when chloride was replaced by gluconate or when bicarbonates were removed from bathing buffers. Although ion transport has not been measured in the presence of bethanecol and carbachol, it can be suggested, based on the above study, that part of the fluid secretion in the presence of these secretagouges could result from chloride secretion.

In the jejunum taken from fed, starved and undernourished gerbils, PGE2 reversed normal absorption into secretion and the amount of fluid secreted as a result of the presence of PGE2 in the starved and undernourished jejunum was significantly higher than in the fed control. The basal fluid absorption in the ileaum taken from fed animals was converted to secretion in the presence of PGE2. Similar to what had happened in the jejunum, fluid secretion in the presence of PGE2 was significantly enhanced in the ileum taken from starved and undernourished gerbils when compared with the ileum of the fed group. Carey, *et al* (1994) showed that a maximal increase in Isc evoked by PGE2 was significantly greater in tissues from fasting piglets compared with those in the fed controls. In conclusion, starvation sensitizes the jejunum and ileum to the effects of bethanecol and carbachol, while starvation and undernourishment sensitize the two regions of the small intestine to the effects of PGE2.

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