An inherent acceleratory effect of insulin on small intestinal transit and its pharmacological characterization in normal mice

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Abstract

**AIM:** To study an inherent effect of insulin on small intestinal transit and to explore involvement of various systems/mechanisms in normal mice.

**METHODS:** Insulin at the doses of 2 µU/kg, 2 mU/kg, 2 U/kg or vehicle was subcutaneously administered to four groups of overnight fasted normal male mice. Blood glucose (BG) levels were measured 2 min before insulin administration and 2 min before sacrificing the animals for the measurement of small intestinal transit (SIT). Charcoal meal was administered (0.3 mL) intragastrically 20 min after insulin administration and animals were sacrificed after 20 min and SIT was determined. For exploration of the various mechanisms involved in insulin-induced effect on SIT, the dose of insulin which can produce a significant acceleration of SIT without altering BG levels was determined. The following drugs, atropine (1 mg/kg), clonidine (0.1 mg/kg), ondansetron (1 mg/kg), naloxone (5 mg/kg), verapamil (8 mg/kg) and glibenclamide (10 mg/kg), were administered intravenously 10 min prior to the administration of insulin (2 µU/kg).

**RESULTS:** The lower doses of insulin (2 µU/kg and 2 mU/kg) produced a significant acceleration of SIT from 52.0% to 70.7% and 73.5% without lowering blood glucose levels (P < 0.01), while the highest dose of insulin (2 U/kg) produced a fall in blood glucose levels which was also associated with significant acceleration of SIT (P < 0.01). After pretreatment of insulin (2 µU/kg) group with atropine, insulin could reverse 50% of the inhibition produced by atropine. In clonidine-pretreated group, insulin administration could reverse only 37% of the inhibition produced by clonidine and inhibition of SIT was significant compared with vehicle + insulin-treated group, i.e. from 74.7% to 27.7% (P < 0.01). In ondansetron-pretreated group, insulin administration could produce only mild acceleration of SIT (23.5%). In naloxone-pretreated group, insulin administration could significantly reverse the inhibition of SIT produced by naloxone when compared with naloxone *per se* group, i.e. from 32.3% to 53.9% (P < 0.01). In verapamil-pretreated group, insulin administration could only partially reverse the inhibition (65%). In glibenclamide-pretreated group, insulin administration produced further acceleration of SIT (12.2%).

**CONCLUSION:** Insulin inherently possesses an acceleratory effect on SIT in normal mice. Adrenergic and cholinergic systems can play a significant role. Calcium channels and opioidergic system can play a supportive role; in addition, enhancement of endogenous insulin release can augment the effect of exogenously administered insulin on SIT.

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**Key words:** Adrenergic system; Blood glucose levels; Ca^{2+} channels; Cholinergic system; Insulin; Intestinal transit; Opioid system; Serotonergic system

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**INTRODUCTION**

Insulin is the drug of choice in the management of elevated blood glucose level in type 1 and sometimes in type 2 diabetes mellitus\(^1\). In addition to its effect on glucose metabolism, insulin is reported to act as a neuromodulator in the central nervous system\(^2\) and as a...
mild analgesic[9]. Takeshita and Yamaguchi[10] characterized an inherent antinociceptive response for insulin when administered subcutaneously in mice. Their study has also shown that the antinociceptive response is independent of hypoglycemic action of insulin.

Gastrointestinal (GI) disorders are common in diabetic patients[3]. About 75% of these patients suffer from GI disorders due to GI neuropathy leading to considerable morbidity[6,7]. These GI disorders include nausea, gastric stasis, constipation, fecal incontinence and diarrhea[8,9]. Clinical studies are available that report insulin therapy increases the gastric emptying through hypoglycemic effect[10,11], but no detailed report is available in normal experimental animals on its effect on small intestinal transit and mechanism involved. In this study, we, therefore, investigated an inherent effect of insulin on small intestinal transit in normal mice and explored the involvement of possible mechanisms. The outcome of this experiment may provide valuable insights into the effect of insulin on normal intestinal transit.

**MATERIALS AND METHODS**

**Animals**

Adequate number of randomly bred normal/healthy adult Swiss albino male mice, weighing between 20-25 g, were obtained from Central Animal House, JIPMER, Pondicherry. One week before the study, the animals were housed at Departmental Animal House in polypropylene cages under standard laboratory conditions. Animals were fasted overnight prior to the experiment in cages with mesh bottom and had free access to water. Experiments were performed during the day time (09:00 to 18:00). The experimental protocol was approved by JIPMER Institutional Animal Ethics committee.

**Drugs and chemicals**

Atropine injection IP (S K Parenterals Pvt. Ltd., Tetali), clonidine HCl (C.H. Boehringer Sohn Ingelhein, Germany), glibenclamide (Hoechst India Ltd., Bombay), gum acacia IP (Hikasu Chemicals, Mumbai), insulin injection IP (purified bovine insulin, 40 U per milliliter; Knoll Pharmaceuticals Ltd., Aslai, India), naloxone HCl (Endo Labs, USA), ondansetron (Cipla Ltd., Mumbai), verapamil (Torrent Laboratories Pvt. Ltd., Ahmedabad), wood charcoal (SD Fine Chemicals, Boisar) were used in this study. All the drugs were dissolved in sodium chloride injection IP except glibenclamide which was dispersed in 50 g/L Tween 80 in water for injection before administration.

**Administration of insulin**

Mice were randomly divided into four groups, each group consisted of 6 mice. Each group was subcutaneously (sc) administered insulin 2 µU/kg, 2 mU/kg, 2 U/kg or vehicle. Blood glucose level was recorded before insulin administration. Charcoal meal was administered 20 min after insulin administration and SIT was determined after 40 min.

**Measurement of small intestinal transit**

The small intestinal transit (SIT) was determined by identifying leading front of intragastrically (ig) administered marker in small intestine of an animal[12,13]. Charcoal meal marker was freshly prepared by dispersing 10 g of wood charcoal in 50 g/L gum Acacia mucilage. After 20 min of insulin administration, each mouse received 0.3 mL of this suspension intragastrically using metallic oral cannula. After 20 min, animals were sacrificed by intravenous administration of sodium pentobarbital (100 mg/kg), abdomen opened, the leading front of marker was identified in the small intestine and tied immediately to avoid movement of marker. The entire length of small intestine was isolated by cutting at pyloric and ileocaecal ends. The distance travelled by the charcoal meal and the total length of the intestine were measured in cm. The SIT was expressed as percentage (%) of the distance travelled by the charcoal meal to length of the intestine. This was carried out in the animals 40 min after insulin administration.

**Measurement of blood glucose**

Blood glucose (BG)[14] was measured by placing a drop of blood obtained by tail venipuncture, over an appropriate glucometric, read by Advantage Glucometer (Boehringer Mannheim Corporation, Indianapolis, USA) and expressed as %, change in the glucose level considering the initial value of that animal as 100. This estimation was done 2 min before insulin/vehicle administration and 2 min before sacrificing the animal for measuring small intestinal transit.

**Mechanisms of insulin-induced acceleration of small intestinal transit**

Insulin at 2 µU/kg dose significantly accelerated (35.7%) small intestinal transit without affecting the blood glucose level; hence this dose was selected to evaluate the mechanism of insulin-induced intestinal hypermotility (Table 1). For exploring the various systems/mechanisms involved in insulin-induced effect on SIT, the antagonists or agonists (agents) of the following systems were attempted (Figure 1).

**Cholinergic system**

Involvement of cholinergic system was evaluated by using a well studied non-specific cholinergic antagonist atropine. The dose selection was based on a study reported by Chaudhuri et al[15]. Atropine (1 mg/kg) was injected intravenously (iv) 10 min before insulin administration (2 µU/kg sc) to one group of animals. After 40 min of insulin administration, the animals were sacrificed to measure SIT. Another group was treated similarly but with vehicle + insulin (Figure 1).

**Adrenergic system**

Involvement of adrenergic system was evaluated by using clonidine (0.1 mg/kg iv). The dose selection was based on studies reported by Donoso et al[16] and DiTullio
agents was evaluated by using naloxone (5 mg/kg iv). The dose selection was based on studies reported by Stein et al.[20] and Peana et al.[21]. Naloxone (5 mg/kg iv) was injected 10 min before insulin administration (2 U/kg sc). After 40 min of insulin injection, the animals were sacrificed to measure SIT (Figure 1).

**Calcium channels**

Involvement of calcium channels was evaluated by using verapamil (8 mg/kg iv). The dose selection was based on studies reported by Ramaswamy et al.[24] and Amos et al.[25]. Verapamil (8 mg/kg iv) was injected 10 min before insulin administration (2 U/kg sc). After 40 min of insulin administration, the animals were sacrificed to measure SIT (Figure 1).

**Insulin secretogogue**

Influence of elevated endogenous insulin levels on SIT was evaluated by using glibenclamide (10 mg/kg iv). The dose selection was based on studies reported by Ramaswamy et al.[24] and Ojewole et al.[27]. Glibenclamide (10 mg/kg iv) was injected 10 min before insulin administration (2 µU/kg sc) to one group of animals. After 40 min of insulin administration, the animals were sacrificed to measure SIT (Figure 1).

**Statistical analysis**

Results are expressed as mean ± SE and analyzed statistically using ANOVA, followed by Dunnett's multiple comparisons test. A P<0.05 was considered statistically significant.

**RESULTS**

**Effect of insulin on small intestinal transit**

Insulin administration at lower doses (2 µU/kg and 2 mU/kg) produced a significant acceleration of SIT without altering the blood glucose levels (P<0.01 Table 1), while the higher dose of insulin (2 U/kg) produced a profound fall in blood glucose levels (P<0.01) which was associated with an acceleration of SIT.

**Cholinergic system**

Atropine (1 mg/kg) per se produced an attenuation of SIT by 47.0% when compared with vehicle treated group. In

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**Table 1 Effect of insulin administration on small intestinal transit and blood glucose levels in normal mice**

| Treatment | Insulin (U/kg sc) | % SIT | Blood glucose (%) |
|-----------|------------------|-------|-------------------|
| Vehicle   |                  |       |                   |
| 2 µ µ     | 52.06 ± 1.48     | 103.73 ± 4.19 |
| 2 m µ     | 70.77 ± 0.62     | 99.47 ± 1.59  |
| 2 µ µ     | 73.53 ± 0.63     | 94.54 ± 4.62  |
| 2 µ µ     | 80.10 ± 1.93     | 42.09 ± 1.78  |

Each value represents the mean ± SE (n=6 sc; subcutaneous; SIT: small intestinal transit; \(^*\)P<0.01 vs vehicle treated group; \(^\dagger\)Values are % SIT considering the total length of the intestine as 100, SIT was determined 40 min after insulin administration; \(^\ddagger\)Blood glucose was estimated 2 min before insulin/vehicle administration and 2 min before sacrificing the animals for the measurement of small intestinal transit (SIT); final blood glucose expressed as % considering the initial blood glucose of each animal as 100.

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**Figure 1 Experimental design**

Carried out to explore various systems/mechanisms involved in insulin-induced acceleration of small intestinal transit in normal mice.

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**Agents**

| Agent/vehicle | Cholinergic system |
|---------------|--------------------|
| 1 Atropine (1 mg/kg iv) |                      |
| 2 Clonidine (0.1 mg/kg iv) | Adrenergic system |
| 3 Ondansetron (1 mg/kg iv) | Serotonergic system |
| 4 Naloxone (5 mg/kg iv) | Opioidergic system |
| 5 Verapamil (8 mg/kg iv) | \(\text{Ca}^2\) channel blocker |
| 6 Glibenclamide (10 mg/kg iv) | Insulin secretogogue |

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atropine-pretreated group, insulin administration (2 µU/kg) could not completely reverse the inhibition produced by atropine. Insulin could overcome only 50% of the inhibition produced by atropine (Table 2 and Figure 2).

**Adrenergic system**
Clonidine (0.1 mg/kg) per se produced an inhibition of SIT by 72.0%. Conversely, in clonidine-pretreated group, insulin administration (2 µU/kg) could partially reverse (37%) the inhibition produced by clonidine but the inhibition of SIT was still significant (P < 0.01) when compared with vehicle + insulin-pretreated group (Table 2 and Figure 2).

**Serotonergic system**
Ondansetron (1 mg/kg) per se could not alter the SIT when compared with vehicle-treated group. Conversely, in ondansetron-pretreated group, insulin administration (2 µU/kg) could produce only mild acceleration of SIT (23.5%) (Table 2 and Figure 2).

**Opioidergic system**
Naloxone (5 mg/kg) per se produced a significant inhibition of SIT by 41.2% when compared with vehicle-treated group. In naloxone-pretreated group, insulin administration (2 µU/kg) could significantly reverse the inhibition produced by naloxone (P < 0.01) when compared with naloxone per se group (66.4%). However, the reversal of inhibition was still significantly lower than insulin per se group (P < 0.01) (Table 2 and Figure 2).

**Calcium channels**
Verapamil (8 mg/kg) per se produced a significant deceleration of SIT by 26.0% when compared with vehicle-treated group. In verapamil-pretreated group, insulin administration (2 µU/kg) could only partially reverse the deceleration produced by verapamil (65%) (Table 2 and Figure 2).

**Insulin secretogogue**
Glibenclamide (10 mg/kg) per se produced a significant acceleration of SIT by 43.8% when compared with vehicle-treated group. In glibenclamide-pretreated group, insulin administration (2 µU/kg) produced a further acceleration of SIT by 12.2% (Table 2 and Figure 2).

**DISCUSSION**
Recent experiments in streptozotocin (STZ)-induced diabetes in rats have demonstrated deleterious effects on the neuromuscular junction as well as on muscle itself. Actions at both sites may contribute to neuropathy and functional alterations in muscle contractile properties.[25,26] Abnormalities in gastric emptying and small intestinal motor function were also reported in STZ-treated rats.[27,28] This may be ascribed to the ability of STZ, which not only destroys β-cells of pancreas leading to diabetic state, but also affect the nervous system function which maintains the tone and motility of GI smooth muscles. Hence, STZ-induced experimental diabetic model became untenable to explore the effect of any agent on SIT, instead normal or healthy animals are appropriate for exploring inherent effect of any substance on SIT. Therefore, the data obtained can reflect the true changes and are devoid of the influence of degenerative changes induced by STZ in laboratory animals.

Our study with insulin administration demonstrated an interesting finding that after 40 min of its administration in normal mice, lower doses of insulin (2 µU/kg and 2 mU/kg) significantly accelerated the small intestinal transit (P < 0.01) without lowering blood glucose levels. On the other hand, the highest dose of insulin (2 µU/kg) produced a significant fall in blood glucose levels which was also associated with an acceleration of SIT (P < 0.01). The available literature indicated that insulin-induced hypoglycemia accelerates gastric emptying of solids and liquids in long-standing type 1 diabetes[29] or stimulates gastric vagal activity causing an increase in gastric emptying in healthy volunteers[30]. These effects on gastric emptying were dependent on blood glucose levels[29,30]. Moreover, these findings were observed with normal or higher doses of insulin. In our experiment, the blood glucose levels were not affected by the lower doses of insulin (2 µU/kg or 2 mU/kg) but produced a significant acceleration of SIT. The sub-hypoglycemic doses of insulin were used to avoid the hypoglycemic effect on intestinal motility. We suggest that the blood glucose levels may not play a significant role in accelerating SIT at least in the lower doses of insulin, indicating an inherent acceleratory effect of insulin on SIT. Our findings are partly in agreement with Takeshita and Yamaguchi[8] who reported antinociceptive effect of insulin was independent of blood glucose level in normal mice. It seems that insulin therapy may bring about an additional benefit in diabetic patients by normalizing the derangement of the gastrointestinal motility.

The gastrointestinal tract is in a continuous state of contraction, relaxation and secretion. These functions are controlled by neurohumoral systems, which in turn are regulated by various receptor systems, such as cholinergic, adrenergic, serotonergic, opioidergic and cell surface channels.[31] Many drugs affect GI transit by acting as agonists or antagonists at specific cellular receptors.[32,33] Acceleration of GI motility can be achieved by direct stimulation of gastrointestinal muscle, by activation of excitatory neural pathways or by inhibition of inhibitory pathways. Deceleration can be produced by direct relaxant effect on smooth muscle, by inhibiting the excitatory neural pathways or by activation of inhibitory pathways. Insulin’s inherent acceleration of SIT can be evaluated by exploring the following systems.

**Cholinergic system**
Atropine is frequently used as a tool for identifying mechanisms involving cholinergic pathways[34]. It is a non-specific competitive antagonist of acetylcholine for muscarinic receptors and abolishes the effects of acetylcholine completely on the GI tract. Both in normal subjects and in patients with GI diseases, full therapeutic doses of atropine (0.5-1 mg) produce definite and prolonged inhibitory effect on the motor activity of the
Table 2 Influence of various systems / mechanisms on insulin-induced acceleration of small intestinal transit in normal mice

| Pretreatment Agent/vehicle (mg/kg iv) | Treatment Insulin (2 µU/kg sc) | % SIT | % Acceleration | % Inhibition |
|-------------------------------------|---------------------------------|-------|----------------|--------------|
| Vehicle                             | Vehicle                         | 55.10 ± 1.46 | --              | --           |
| Vehicle                             | Insulin                         | 74.76 ± 1.40  | 35.68           | --           |
| Atropine (1)                        | Vehicle                         | 29.19 ± 3.28  | --              | 47.02        |
| Atropine (1)                        | Insulin                         | 37.32 ± 3.58  | --              | 50.08        |
| Clonidine (0.1)                     | Vehicle                         | 15.50 ± 1.46  | --              | 71.86        |
| Clonidine (0.1)                     | Insulin                         | 27.71 ± 4.78  | --              | 62.93        |
| Ondansetron (1)                     | Vehicle                         | 51.69 ± 1.13  | --              | 6.18         |
| Ondansetron (1)                     | Insulin                         | 63.84 ± 5.88  | --              | 14.60        |
| Naloxone (5)                        | Vehicle                         | 32.39 ± 2.56  | --              | 41.21        |
| Naloxone (5)                        | Insulin                         | 53.90 ± 2.95  | --              | 27.90        |
| Verapamil (8)                       | Vehicle                         | 40.72 ± 1.72  | --              | 26.09        |
| Verapamil (8)                       | Insulin                         | 48.61 ± 3.27  | --              | 34.97        |
| Glibenclamide(10)                   | Vehicle                         | 79.23 ± 1.32  | 43.79           | --           |
| Glibenclamide(10)                   | Insulin                         | 88.88 ± 3.52  | 18.88           | --           |
| Glibenclamide(10)                   | Glibenclamide (10)              | 88.88 ± 3.52  | 61.30           | --           |

Each value represents the mean ± SE (n=6) or %.  
1P<0.01 vs vehicle + vehicle group.  
2P<0.01 vs vehicle + insulin group.  
3compared with vehicle + vehicle group.  
4compared with vehicle + insulin group.

stomach, duodenum, jejunum and ileum. Our study also confirmed inhibitory effect of atropine on SIT in normal mice. When atropine-injected group (1 mg/kg) was treated with insulin (2 µU/kg), insulin failed significantly to reverse the inhibition of SIT induced by atropine when compared with insulin per se treated group (Table 2). This finding indicates that insulin acts directly through muscarinic receptors to accelerate the SIT. Since clonidine could not completely produce acceleratory effect by some other pathways in addition to cholinergic pathways as atropine could not completely prevent the acceleratory effect of insulin (Figure 2).

Adrenergic system

Clonidine has presynaptic α2 receptor agonistic activity. Stimulation of α2 receptors which are present on excitatory cholinergic intramural neurons in the intestine, attenuates the release of acetylcholine presynaptically, thereby producing depression of intestinal motility. In our study, we observed a significant attenuation of SIT in clonidine (0.1 mg/kg) per se treated group when compared with vehicle-treatead group (P<0.01), thus confirming the inhibitory effect of clonidine on SIT in mice.

When clonidine-injected group (0.1 mg/kg) was treated with insulin (2 µU/kg), the SIT was inhibited by 63% as compared with vehicle + insulin-treated group (Figure 2), thereby indicating that even though insulin could partially reverse the SIT (37%), the inhibitory effect of clonidine was still significant when compared with insulin per se treated group (P<0.01) (Table 2). This finding indicates α-adrenergic pathways may be dominant in producing acceleratory effect of insulin on SIT. Insulin might have interfered with presynaptic α2 receptors and facilitated the release of acetylcholine from excitatory neurons in the intestine. Since clonidine could not completely inhibit the SIT, we suggest that in addition to α-adrenergic pathways, insulin action may be associated with any other pathways but playing a minor role.

Serotonergic system

5-Hydroxy tryptamine (5-HT) receptors are broadly classified into five subtypes: 5-HT1, 5-HT2, 5-HT3, 5-HT4 and 5-HT7. Of these principal receptors, 5-HT1 receptor has been suggested to play a role in the regulation of gastrointestinal motor function in many animal species and humans. In the mouse ileum, 5-HT1 receptors modulate neuronal activity within the enteric nerves leading to the contraction of the smooth muscle. The selective antagonists of 5-HT1 receptors block the depolarizing actions of 5-HT on vagal afferents in gastrointestinal tract and have been proved to be a major breakthrough in the control of chemotherapy and radiotherapy-induced emesis. In our study, a selective 5-HT1 receptor antagonist, ondansetron (1 mg/kg), per se could not alter the normal SIT. Indeed, we selected the dose of ondansetron (1 mg/kg) which had significantly increased whole gut transit time in mice. On the other hand, in ondansetron-treated group, insulin administration (2 µU/kg) slightly accelerated SIT (23.5%) (Table 2). This finding indicates that at this dose, ondansetron might rather partially inhibit the insulin-induced acceleration on SIT (14.6%) (Figure 2). Ondansetron (1 mg/kg) might have a prominent effect on the large intestine of mice. Hence, we propose that dose-
dependent studies are required to show the involvement of serotonergic systems with ondansetron in small intestinal motility and to evaluate insulin’s acceleratory effect on SIT in this animal model.

**Opioidergic system**

Opioid neurons constitute the largest population of peptide-containing neurons in the myenteric plexus of the gut\[42\]. Opioid receptors are present on enteric nerves, epithelial cells and muscle cells\[43,44\]. It is known that opioid agonists slow intestinal transit particularly at the proximal portion and also reduce luminal secretions\[45\]. The administration of naloxone will antagonize or reverse the actions of opioids and of similar agents\[46\]. Therefore, the use of naloxone is expected to produce normal SIT, followed by administration of any opioid. In contrast, we observed that naloxone (5 mg/kg) per se produced significant attenuation of SIT (P < 0.01) in this animal species. This finding indicates that naloxone acts similar to opioids (Table 2).

In support of this finding, we came across similar contrasting reports but on gastric emptying. Champion *et al*\[46\] reported that naloxone (2 mg) delayed gastric emptying of radio-opaque material in healthy volunteers. They suggested that naloxone inhibited endogenous opiate system which normally stimulates gastric emptying and they had used the dose of naloxone two to three times greater than those usually given to reverse narcotic-induced respiratory depression and in large doses, naloxone itself may inhibit gastric emptying. Similarly, Asai and Power\[47\] also reported naloxone (0.01-10 mg/kg) per se significantly inhibited gastric emptying in rats. These studies revealed effect of naloxone on gastric emptying, but our study reveals a similar finding in the small intestine. Our study with naloxone per se may also suggest that naloxone may inhibit a subset of endogenous opiate system which normally stimulates movement of the contents of the intestine in this animal species. We used higher dose of naloxone to block all the receptors, thereby masking the effects of endogenous opioids, and to explore whether any subset of opioid receptors were involved in insulin-induced acceleration of SIT.

When naloxone-injected group (5 mg/kg) was treated with insulin, the SIT was significantly reversed as compared with naloxone per se treated group (P < 0.01) (Table 2). However, the naloxone-induced inhibition of SIT (28%) in insulin-treated group was still significant when compared with insulin per se treated group (Figure 2). This finding suggests that insulin may act through a subset of opioid receptors in the intestine to accelerate the SIT and that might be inhibited by naloxone treatment.

**Calcium channels**

Calcium is involved in the initiation of contraction of smooth muscle\[48\]. The visceral smooth muscle has a poorly developed sarcoplasmic reticulum and the increase in intracellular calcium concentration is primarily due to Ca\(^{2+}\) influx from the extracellular fluid via voltage-gated Ca\(^{2+}\) channels\[49\]. The L-type calcium channel is present in many cells and it is the main source of Ca\(^{2+}\) for contraction of smooth muscle\[48\]. This channel is blocked by dihydropyridines such as nifedipine, and other drugs such as verapamil and diltiazem\[50\]. Verapamil, a phenylalkylamine derivative, blocks the calcium channels on the surface of smooth muscle cells and relaxes the smooth muscle, thereby attenuating the intestinal motility\[45,51\]. The objective of this experiment was to evaluate the involvement of Ca\(^{2+}\) in insulin-induced acceleration of SIT. Verapamil at the given dose (8 mg/kg) per se significantly inhibited SIT, indicating the involvement of Ca\(^{2+}\) channels in normal physiology of small intestinal motility (Table 2). In verapamil-pretreated group, insulin administration could reverse the inhibition produced by verapamil by 65%. This finding may indicate that as insulin could not completely reverse inhibition of calcium channels, some other systems are also involved in acceleratory effect of insulin action on SIT.

**Insulin secretagogue**

Glibenclamide, an oral hypoglycemic drug, acts by stimulating insulin release from β-cells of pancreas\[1\]. The objective of this experiment was to find whether endogenously released insulin can potentiate the action of exogenously administered insulin effect on SIT. Glibenclamide (10 mg/kg) per se produced a significant acceleration of SIT by 43.8% (Table 2). This finding may indicate that endogenous release of insulin by glibenclamide increases the SIT.

When insulin-injected group was pretreated with glibenclamide, the SIT was further accelerated by 12% (Figure 2). This observation indicated that insulin from endogenous sources might have contributed to the additional acceleration of SIT by exogenously administered insulin.

Figure 2 indicates the significant involvement of following systems in decreasing order of acceleratory effect of insulin on SIT: adrenergic system > cholinergic system > calcium channels > opioidergic system (P < 0.01). In addition, release of endogenous insulin augments the effect of exogenously administered insulin on SIT.

In conclusion, the sub-hypoglycemic doses (2 µU/kg or 2 mU/kg) of insulin accelerate the small intestinal transit in normal mice without markedly changing blood glucose levels. It can be assumed that therapy of type 1 diabetes with insulin can simultaneously relieve at least one of the diabetic GI complications, such as constipation or sometimes may aggravate the diabetic neuropathy-associated diarrhoea. Furthermore, we explored influence of various systems, channels and endogenous insulin in acceleratory effect of exogenously administered insulin. Based on these observations, we report that adrenergic and cholinergic pathways play a significant role in hypermotility of small intestine induced by insulin administration in mice. Calcium channels and opioidergic pathways play supportive role; in addition, enhancement of endogenous insulin release can augment the effect of exogenously administered insulin on SIT.

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