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Enhancement of nasal absorption of insulin and calcitonin using polyacrylic acid gel

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The effects of polyacrylic acid gel on the nasal absorption of insulin and [Asu^{1,7}]-eel calcitonin were investigated in rats. The nasal administration of insulin (1 IU kg^{-1}) in polyacrylic acid gel at 0·1 and 1% w/v showed maximum hypoglycaemic effects at 30 min and 1 h after administration, respectively. However, the nasal administration of insulin in carboxymethyl cellulose (1% w/v) solution had no hypoglycaemic effect at the same dose. When [Asu^{1,7}]-eel calcitonin (10 U kg^{-1}) was administered nasally in polyacrylic acid gel (0·1% w/v), a prominent hypocalcaemic effect was observed during the first 30 min. Nasal administration of [Asu^{1,7}]-eel calcitonin in saline had no hypocalcaemic effect at the same dose. The results indicate that the polyacrylic acid gel base significantly enhanced the absorption of insulin and [Asu^{1,7}]-eel calcitonin via the nasal cavity.

The intranasal route for drug administration is probably one of the most neglected methods for distributing drugs to the systemic circulation. Recently, however, evidence has suggested that this route may be useful for drugs hitherto only administered parenterally. For example, the nasal administration of insulin to dogs resulted in a significant increase in blood immunoreactive insulin levels with a remarkable hypoglycaemia (Hirai et al 1978). Moreover, the nasal absorption of insulin was enhanced by the addition of adjuvants such as surfactants to the insulin solution (Hirai et al 1981).

Meanwhile, the aqueous gel base of polyacrylic acid, which is a group of carboxyvinyl polymers cross-linked with allyl sucrose, was found to improve the absorption of peptide hormones such as insulin (Morimoto et al 1980) and calcitonin (Morimoto et al 1984) when administered rectally.

The present study was designed to evaluate the feasibility of intranasal administration of insulin and [Asu^{1,7}]-eel calcitonin using a polyacrylic acid aqueous gel (Carbopol gel). The absorption of insulin and [Asu^{1,7}]-eel calcitonin through nasal mucosa was estimated from the reduction in plasma glucose and plasma calcium, respectively.

Materials and methods

Materials. Bovine crystalline insulin (Sigma Chem. Co., Mo., USA) and [Asu^{1,7}]-eel calcitonin (Toyo Jozo Co., Japan), a semisynthetic analogue of eel calcitonin in which the S–S bond at the first and seventh amino acid in the eel calcitonin molecule is replaced by a CH_2–CH_2 bond, was used. A polyacrylic acid (Carbopol 941) was obtained from B. F. Goodrich Chem. Co., Oh, USA. All reagents were of analytical reagent grade.

Preparations. Polyacrylic acid aqueous gel base was prepared by presoaking polyacrylic acid in distilled water for 15 h at room temperature (20 °C), and adding 10% NaOH solution to adjust the pH. The final concentration of gel was adjusted by the addition of water as described by Morimoto et al (1980). The concentrations of polyacrylic acid in the gel bases were 0·1 and 1% w/v and the pH values selected for the study were 4·5, 5·5 and 7·5. Insulin was suspended in the gel base and [Asu^{1,7}]-eel calcitonin was dissolved in the gel base. The preparations were stored in the dark at 6 °C.

Animal experiments. For insulin administration, adult Wistar strain male rats, 200–250 g, were used, and for [Asu^{1,7}]-eel calcitonin, young Wistar strain male rats, 80–100 g, were selected. The animals were fasted for 20 h before experiments and were anaesthetized by intraperitoneal injection of sodium pentbarbitone at a dose of 50 mg kg^{-1}. The surgical operation was carried out on the rats in the same way as described by Hirai et al (1981). After an incision had been made in the neck, the trachea was cannulated with a polyethylene tube. Another tube was inserted from the oesophagus to the posterior part of the nasal cavity for the administration of the preparation. The nasopalatine was closed with an adhesive agent to prevent drainage of the drug from the nasal cavity to the mouth. The preparation was administered to the nasal cavity through the tube by means of a syringe at a volume of 0·05 ml kg^{-1} for insulin and of 0·025 ml kg^{-1} for [Asu^{1,7}]-eel calcitonin. In comparative studies, insulin in carboxy methyl cellulose (CMC) solution (1% w/v) and [Asu^{1,7}]-eel calcitonin in 0·9% NaCl (saline) were also prepared and administered nasally to separate groups of rats. Blood samples (0·2 ml) were obtained with a heparinized syringe from a femoral vein 10 min before and at 30 min and 1, 2 and 3 h after administration of the preparations.

Analytical method. Plasma was separated by centrifugation at 3000 rev min^{-1} and plasma glucose was determined by the glucose oxidase/peroxidase method (Kiang 1976). Plasma calcium was determined by *o*-cresolphthalain complexone method (Morin 1974).

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Results and discussion

Administration of insulin. Fig. 1 shows the effects of two different concentrations of polyacrylic acid in the gel base (pH 6·5) on plasma glucose concentrations after nasal administration of insulin at a dose of 1 IU kg^{-1}. The time course of hypoglycaemic effects after nasal administration showed varying patterns with two concentrations of polyacrylic acid. With the 0·1% w/v gel base, the maximum hypoglycaemic effect was obtained 30 min after the administration, while with the 1% w/v gel base, the plasma glucose levels decreased slowly and the maximum hypoglycaemic effect was obtained at 1 h. In a comparative study, nasal administration of insulin in CMC (1% w/v) solution produced no hypoglycaemic effect at the dose of 1 IU kg^{-1}. The nasal administration of polyacrylic acid gel (0·1% w/v, pH 6·5) alone did not have any hypoglycaemic effect.

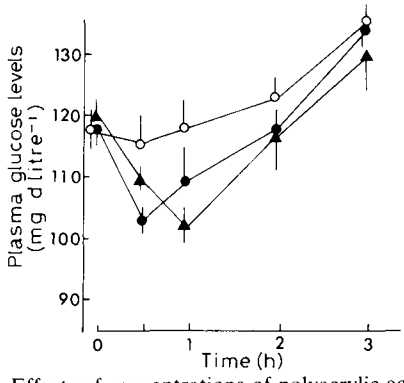

FIG. 1. Effects of concentrations of polyacrylic acid in the gel bases (pH 6·5) on changes in plasma glucose levels after nasal administration of insulin (1 IU kg^{-1}) in rats. ● 0·1% w/v PAA gel, ▲ 1% w/v PAA gel, ○ CMC solution (1% w/v). Each point represents a mean ± s.e.m. of five animals.

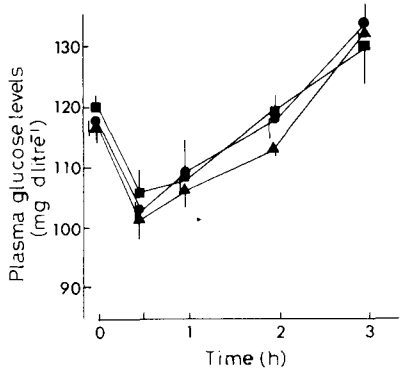

FIG. 2. Effects of pH of polyacrylic acid gel bases (0·1% w/v) on changes in plasma glucose levels after nasal administration of insulin (1 IU kg^{-1}) in rats. ● pH 4·5 PAA gel, ▲ ph 6·5 PAA gel, ■ pH 7·5 PAA gel. Each point represents a mean ± s.e.m. of five animals.

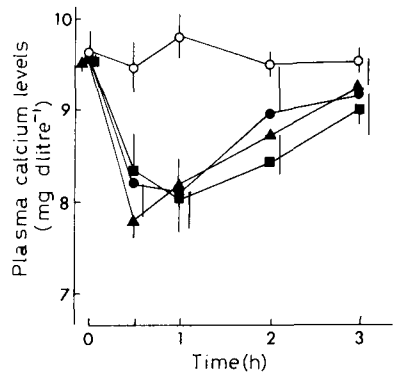

FIG. 3. Effects of pH of polyacrylic acid gel bases (0·1% w/v) on changes in plasma calcium levels after nasal administration of [$Asu^{1,7}$]-eel calcitonin in young rats. ● pH 5·5 PAA gel, ▲ pH 6·5 PAA gel, ■ pH 7·5 PAA gel, ○ saline solution. Each point represents a mean ± s.e.m. of five animals.

Fig. 2 shows the effects of pH of polyacrylic acid gel (0·1% w/v) on the absorption of insulin following the nasal administration of insulin at the dose of 1 IU kg^{-1} in rats. The nasal absorption of insulin in terms of change in glucose levels was not influenced by the pH of the gel base.

Administration of [$Asu^{1,7}$]-eel calcitonin. Fig. 3 shows the effects of pH of polyacrylic acid gel (0·1% w/v) on plasma Ca levels after the nasal administration of [$Asu^{1,7}$]-eel calcitonin (10 U kg^{-1}) gel preparations to young rats. With three different pH values of polyacrylic acid gel, all the plasma Ca levels decreased rapidly but the maximum hypocalcaemic effect was obtained at 30 min for pH 6·5 and at 1 h for pH 5·5 and pH 7·5. On the other hand, the nasal administration of [$Asu^{1,7}$]-eel calcitonin in a saline solution at 1 U kg^{-1} produced no hypocalcaemic effects.

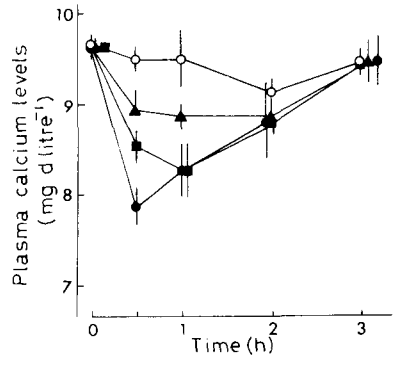

FIG. 4. Dose-dependency of plasma calcium levels following nasal administration of [$Asu^{1,7}$]-eel calcitonin in polyacrylic acid gel base (0·1% w/v, pH 6·5) in young rats. △ 0 U kg^{-1}, ▲ 1 U kg^{-1}, ■ 5 U kg^{-1}, ● 10 U kg^{-1}. Each point represents a mean ± s.e.m. of five animals.

Fig. 4 shows the response profiles of plasma Ca levels at three dose levels following the nasal administration of [Asu^{1,7}]-eel calcitonin in the polyacrylic acid gel (0·1% w/v, pH 6·5). A rapid decrease of the plasma Ca levels was observed after doses of 5 and 10 U kg^{-1} of [Asu^{1,7}]-eel calcitonin, while at the 1 U kg^{-1} there was a slight hypocalcaemic effect.

It has been generally considered that peptide hormones such as insulin and [Asu^{1,7}]-eel calcitonin are poorly absorbed through the mucosal membrane. These results clearly reveal that the absorption of insulin and [Asu^{1,7}]-eel calcitonin from the nasal cavity was significantly enhanced when polyacrylic acid gel was used as vehicle. As after nasal administration of insulin its absorption from 0·1% w/v polyacrylic acid gel is greater than with 1% w/v gel there would seem to be an optimum concentration and possibly an optimum viscosity for the polyacrylic acid gel base. Hirai et al (1978, 1981) reported that the absorption of insulin from the nasal cavity was influenced by pH, being minimal at pH 5·5 and 7·5, near to the isoelectric point and rising sharply on either side. In this study, however, the pH of polyacrylic acid gel did not affect the absorption of insulin from the gel preparation and these results were also consistent with the rectal administration of insulin in polyacrylic acid gel in rats (Morimoto et al 1980). Furthermore, the nasal absorption of [Asu^{1,7}]-eel calcitonin was not affected by the pH of polyacrylic acid gel in the pH range of 5·5–7·5, a result that did not agree with that of the rectal absorption of [Asu^{1,7}]-eel calcitonin in polyacrylic acid gel reported by Morimoto et al (1984).

It is well known that the nasal mucosa has many secretory glands, and water transport through such secretory glands may occur (Kotani et al 1983). A preliminary experiment showed that the polyacrylic acid gel increased the water influx in rat rectum (not published). Therefore, it is considered that the water absorption promoted by this gel base would increase the absorption of insulin and calcitonin from nasal mucosa. On the other hand, the microscopic examination of histological samples and the membrane effects (protein release) showed no damage to the surface of the mucosa by the polyacrylic acid gel basa (Morimoto et al 1980, 1984).

The mechanism of enhancement of nasal absorption of insulin and calcitonin, hydrophilic and macromolecular compounds, by polyacrylic acid gel is unclear. Its main absorption pathway, perhaps, is via paracellular routes through the intracellular channels with water influx rather than via the transcellular route through the epithelial cells.

In conclusion, it has been demonstrated that polyacrylic acid gel may be used as an aqueous vehicle for nasal administration producing a clear enhancement of the absorption of the polypeptide drugs.

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