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Effect of enamine derivatives on the rectal absorption of insulin in dogs and rabbits

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Enamine derivatives resulting from the reaction between ethyl acetoacetate and amino acids were found to promote rectal absorption of insulin in normal rabbits, with the enamine of sodium DL-phenylalanate being the most effective adjuvant tested. Results of glucose tolerance tests in fasted alloxan diabetic rabbits showed that after oral administration of glucose, insulin suppositories containing enamines derived from either sodium phenylalanine or leucine were able to lower the serum glucose level to within the normal range for more than 2·5 h. The absorption-promoting effect of the sodium DL-phenylalanate enamine on rectal absorption of insulin in dogs was studied in two different formulations, gelatin microenema and suppository. The gelatin microenema containing the insulin solution caused a greater increase in the plasma insulin level than that obtained after administration of an insulin suppository.

Insulin is clinically administered parenterally because of poor absorption and degradation via other potential routes of delivery. Rectal or oral administration would offer advantages in terms of convenience to the patient as well as possibly fewer problems with antigenicity (Yamasaki et al 1981). Attempts to promote rectal absorption of insulin have included the use of surfactants (Touitou et al 1978; Nishioka & Kawamura 1978; Schichiri et al 1978). However, surfactants often cause rectal bleeding or other damage to the rectal mucosa. Our laboratories have obtained favourable results in the delivery of insulin with the rectal administration of insulin microenemas containing adjuvants such as salicylate and 5-methoxysalicylate (Nishihata et al 1980, 1981).

In an earlier paper (Kamada et al 1981), the promoting effects of phenylglycine enamines of various β-diketones on the rectal absorption of insulin in rabbits and dogs were reported. Among the β-diketones studied, ethyl acetoacetate was considered to be more advantageous because of its low toxicity (unpublished data). It has also been officially approved as a food additive.

In this paper, the adjuvant efficacy of enamine derivatives in promoting the rectal absorption of insulin in rabbits was studied using enamines derived from various amino acids. In a separate set of insulin absorption experiments in dogs, the enamine derivative of phenylalanine was administered as two different formulations, a gelatin microenema and a suppository, in order to determine if formulation design has any effect on insulin bioavailability.

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MATERIALS AND METHODS

Enamine derivatives of amino acids tested were synthesized according to Dane & Docker (1965). Commercially available crystalline beef insulin (zinc content 0·5% w/w on dry basis, 24·3 i.u. mg^{-1}) (Commonwealth Serum Laboratories, Australia) was used. Other reagents were of analytical grade.

Preparation of suppositories or microenemas

Suppositories were prepared by melting suppository base (Witepsol H-15) (Dynamit Novel Chemicals, Troisdorf-Oberlat, West Germany) at 40 °C on a hot plate and adding the insulin and adjuvant to the melt. The molten mass was poured into disposable plastic molds (Nichi Packing Co, Ltd, Osaka, Japan). Suppositories were kept at 4 °C. Insulin microenemas were prepared using a 4% gelatin solution.

In-vivo studies

Male white rabbits, 3·0 to 3·5 kg, and Beagle dogs, 10 to 11 kg, were fasted (with water available) for 16 h before the experiments. After administration of suppositories, blood samples were taken from the ear vein of rabbits and from the jugular vein of dogs. Blood samples were then centrifuged at 3000 rev min^{-1} for 5 min.

Preparation of alloxan diabetic rabbits

Alloxan diabetic rabbits were prepared and checked following the methods of Nishihata et al (1978).

Glucose tolerance test

Fifteen minutes after the administration of an insulin suppository, a volume of 10 ml kg^{-1} body weight of a 30% glucose aqueous solution was orally administered to rabbits fasted 16 h with water available.

Analytical methods

The serum glucose level was determined by the *o*-toluidine-boric acid method (Nishihata et al 1978) which employs a Glucose Test Kit (Wako Pure Chemical Co, Ltd, Japan). The serum insulin level was determined by radioimmunoassay method described by Nishihata et al (1978) which uses the Phadebas Insulin-Test Kit (Daiichi Radioisotope Lab, Ltd, Japan).

RESULTS AND DISCUSSION

Rabbit study

Rectal administration of a 0·5 g insulin suppository containing 3 i.u. of insulin and 25 mg of the ethyl acetoacetate enamine of sodium phenylalanine (Phe-EtAA Na) caused a rapid increase in the serum insulin level and a concurrent decrease in the serum glucose level in normal rabbits (Fig. 1). The insulin suppositories containing 50 mg L-leucine, 50 mg alanine, 100 mg isoleucine or 100 mg glycerine enamines had a similar effect on the insulin and glucose levels. Table 1 shows the significant decrease in serum glucose levels in rabbits after rectal administration of insulin suppositories containing enamine derivatives as adjuvants.

Of all enamine derivatives examined, the enhancing action of Phe-EtAA Na appeared to be the strongest since the Phe-EtAA Na dosage required to effect the drop in serum glucose was lower than the other enamine dosages (Table 1). A comparison of the enhancing effects of Phe-EtAA Na and phenylglycyl ethyl acetoacetate (PG-EtAA Na), an

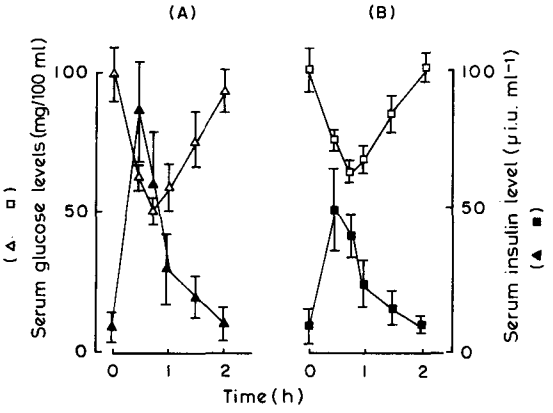

FIG. 1. Plasma levels of glucose (△ and □) and insulin (▲ and ■) in rabbits following rectal administration of 0·5 g suppositories containing 3 i.u. of insulin and (A) 25 mg of Phe-EtAA Na or (B) 25 mg of Leu-EtAA Na. The error bars represent the standard deviation (n = 6).

enamine examined by Kamada et al (1981), showed the serum insulin and glucose levels obtained after rectal administration to be similar. However, the enamine Phe-EtAA Na is less toxic than PG-EtAA Na since the LD50 value of Phe-EtAA Na after i.v. injection in rats is about 1500 mg kg^{-1} while that of PG-EtAA Na is about 740 mg kg^{-1} (unpublished data, Nishihata).

The enamine-enhancement of rectal absorption of insulin in rabbits was found to be dose-dependent. Table 2 shows that increasing the dose of Phe-EtAA Na or Ala-EtAA Na in the suppository up to an enamine dose of 100 mg 0·5 g^{-1} suppository caused an increase in the rabbit insulin plasma peak concentrations after suppository administration.

To clarify the effectiveness of insulin-enamine suppositories in reducing glucose levels, insulin

Table 1. Plasma glucose levels (mg/100 ml) in rabbits after rectal administration of insulin suppositories (3 i.u.) containing enamine derivatives of various amino acids: glycine (Gly-EtAA Na), alanine (Ala-EtAA Na), phenylalanine (Phe-EtAA Na), leucine (Leu-EtAA Na) or isoleucine (Ile-EtAA Na) (n = 6).

	Enamines (mg/0·5 g suppository)				
Time (h)	Gly-EtAA Na (100 mg)	Ala-EtAA Na (50 mg)	Phe-EtAA Na (25 mg)	Leu-EtAA Na (50 mg)	Ile-EtAA Na (100 mg)
0	118·3 ± 13·6	106·5 ± 16·2	101·2 ± 10·2	103·5 ± 8·0	111·3 ± 18·4
0·5	65·2 ± 2·8	66·4 ± 8·2	59·3 ± 5·8	71·8 ± 4·1	68·5 ± 11·3
0·75	54·3 ± 4·9	55·7 ± 6·1	51·2 ± 4·3	60·3 ± 3·8	56·2 ± 7·6
1·0	69·8 ± 11·9	64·5 ± 6·3	56·7 ± 8·6	64·2 ± 4·8	57·5 ± 10·2
1·5	83·7 ± 10·0	72·7 ± 7·2	72·3 ± 10·8	81·8 ± 5·4	69·8 ± 8·7
2·0	97·6 ± 5·3	86·3 ± 8·7	94·3 ± 7·4	101·3 ± 4·6	84·3 ± 6·6
2·5		101·4 ± 11·6			
3·0	108·5 ± 12·7	102·8 ± 12·3	95·8 ± 17·6	107·6 ± 15·8	98·4 ± 13·1

suppositories, containing Phe-EtAA Na or Leu-EtAA Na as adjuvant, were administered to alloxan diabetic rabbits that had been fasted before the experiments and which had a plasma glucose level ca 100 mg/100 ml compared with unfasted alloxan diabetic rabbits whose glucose levels were more varied, ranging between 200–300 mg/100 ml. As shown in Fig. 2, the results of glucose tolerance tests indicate that after oral glucose administration, the insulin suppositories with enamines were able to maintain normal (~100 mg/100 ml) glucose levels for more than 2·5 h. However, insulin suppositories administered without an enamine adjuvant failed to maintain normal glucose levels. The trend shown in Fig. 2 was present in all four rabbits examined.

We previously reported (Nishihata et al 1980, 1982) that salicylate could promote the rectal absorption of insulin and other low lipophilic drugs in rat and dog. In another paper (Nishihata et al 1983), we have demonstrated that acetoacetate esters of glycerol also enhanced the rectal insulin absorption in rabbits. It is well known that both salicylate and acetoacetic acid are able to chelate with divalent metal ions, although less strongly so than EDTA. Kamada et al (1981) found the acetoacetate enamine of phenylglycine chelated with calcium acted as a promoting adjuvant on rectal-insulin absorption while 3-acetylbutyrolactone enamine, an enamine that does not chelate with calcium, did not enhance

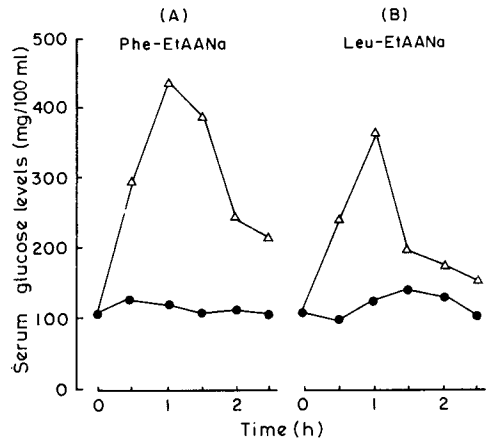

FIG. 2. Effect of insulin suppositories (3 i.u.) containing (A) 25 mg of Phe-EtAA Na (●) or (B) 25 mg of Leu-EtAA Na (●) on the plasma glucose levels in fasted alloxan diabetic rabbits employed in the glucose tolerance test. (△) represents the results of control experiments where only oral glucose was administered. The suppositories were given 15 min before the glucose tolerance test.

Table 2. Dose-dependency of the promoting action of enamines on the rectal absorption of insulin administered as suppositories to rabbits and as 4% gelatin microenemas to dogs.

Enamine dose (mg/0·5 g suppository or mg/1·0 g microenemas)	Peak insulin levels (μi.u. ml⁻¹)				
	Rabbits (3 i.u.)				Dogs (12 i.u.)
	Phe-EtAA Na	Phe-EtAA Na with calcium	Ala-EtAA Na	Ala-EtAA Na with calcium	Phe-EtAA Na
0	>10	—	>10	—	>10 (n = 4)
5	>10 (n = 6)	—	>10 (n = 6)	—	—
10	32·5 ± 15·6[a] (n = 4)	>10	19·8 ± 6·3 (n = 4)	>10[b] (n = 4)	—
25	88·3 ± 17·8[a] (n = 6)	30·5 ± 10·9[b] (n = 4)	49·3 ± 11·3[a] (n = 4)	>10[b] (n = 4)	—
50	193·2 ± 28·5[a] (n = 4)	68·3 ± 16·8[b] (n = 4)	78·6 ± 21·3[a] (n = 6)	—	—
75	—	—	—	—	48·5 ± 11·3[a] (n = 4)
100	296·4 ± 47·8[a] (n = 4)	—	168·8 ± 26·5[a] (n = 4)	57·6 ± 21·3[b] (n = 4)	—
150	—	—	—	—	108·9 ± 23·1[a] (n = 4)
300	—	—	—	—	165·3 ± 36·8[a] (n = 4)
400	—	—	—	—	172·8 ± 28·5[a] (n = 4)

[a] $P < 0·005$ versus the plasma insulin levels after rectal administration without enamine.
[b] $P < 0·005$ versus the plasma insulin levels after rectal administration with adjuvant but without calcium gluconate (10 mg/0·5 g suppository).

rectal-insulin absorption. We found in the present study that the enhancing action of enamines on rectal absorption of insulin was inhibited by the presence of calcium gluconate in the microenema (Table 2). Our results appear to indicate that the absorption-promoting efficacy of enamine derivatives on rectal insulin absorption may depend on the ability of the enamine to form a complex with a component such as calcium in the surface membrane thereby altering the permeability of the membrane to insulin.

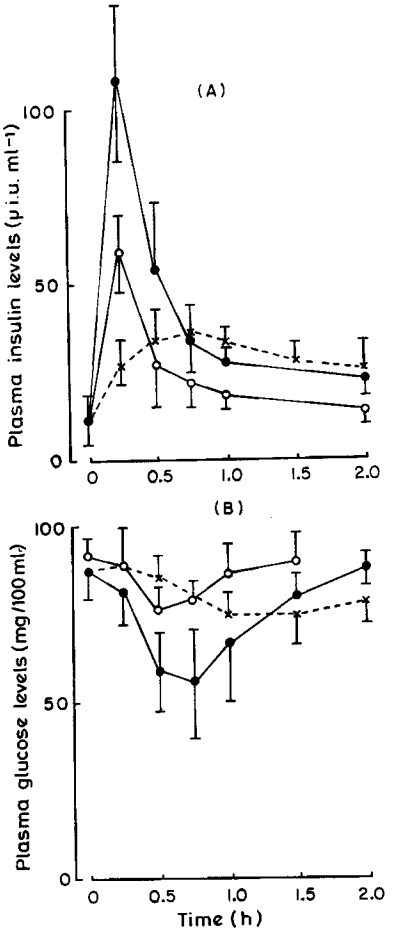

FIG. 3. Plasma levels of (A) insulin and (B) glucose in dogs following intramuscular injection of 2 i.u. of insulin (X); rectal administration of 1 g suppositories containing 12 i.u. of insulin and 150 mg of Phe-EtAA Na in Witepsol H-15 (○); or rectal administration of 1 g microenemas containing 12 i.u. of insulin and 150 mg of Phe-EtAA Na in 4% gelatin (●). The error bars represent the standard deviation (n = 4).

Dog study

The effect of Phe-EtAA Na as absorption adjuvant was also studied in dogs. After rectal administration of an insulin suppository containing 12 i.u. of insulin and 50 mg of Phe-EtAA Na, the serum insulin level significantly decreased as shown in Fig. 3. Maximum serum insulin occurred within 30 min and was followed by a rapid drop in concentration. Concurrent with the increase, serum glucose levels decreased and reached a minimum at about 60 min. However, administration of insulin suppositories without Phe-EtAA Na did not cause increased serum insulin levels and did not change the serum glucose levels.

In comparison to the insulin serum level after i.m. injection, the bioavailability of insulin after rectal administration of an insulin suppository was lower (Fig. 3). A study using a 4% gelatin microenema containing insulin and Phe-EtAA Na resulted in serum insulin levels which were higher and longer lasting than those obtained after the administration of an insulin suppository (Fig. 3). One possible explanation for the lower insulin bioavailability after suppository administration could be the slow rate of insulin solubility in the rectal secretory fluid. If improved suppository formulations can be developed, insulin suppositories containing the enamine derivatives of ethyl acetoacetate and amino acids as adjuvant may have the potential to be effective in the treatment of diabetic patients.

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