fragmentation pathways are shown in Fig. 2. The mass spectrum of the metabolic product, which is appreciably different from the parent compound (Ia), suggests that the product was Ib.

With benzylpiperazine (Ic) the original substrate only could be recovered from the incubation mixture.

No 'in-vitro' degradation of the piperazine ring could be demonstrated in the present study and it is of interest that ring degradation products were found only in the tissues of rats after their being given substituted piperazines for 3–7 days (Breyer 1972; Gaertner & Breyer 1972).

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Effect of aprotinin on the rectal delivery of insulin

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Aprotinin is a polypeptide of 58 amino acids obtained from the lungs or parotid glands. Because of its broad spectrum of protease inhibition, aprotinin has been clinically used in the treatment of chronic urticaria (Berova et al 1974) and other disorders associated with increased protease activity (Franceschini 1970; Amris & Scand 1966). Aprotinin has recently been shown to enhance the absorption of a number of polypeptide hormones (Parsons et al 1979; Philippe et al 1979) including insulin (Freidenberg et al 1981) when administered with these compounds either subcutaneously or intramuscularly. However, after subcutaneous or intramuscular injection of aprotinin alone, insulin levels in the blood have been shown to remain unchanged (Berger et al 1980). Another study has demonstrated that aprotinin can inhibit the degradation of insulin in adipose tissue in-vitro (Paulsen et al 1979).

In earlier papers (Nishihata et al 1981a,b; Kamada et al 1981), the enhancing effect of non-surfactant adjuvants such as salicylate, 5-methoxysalicylate and enamine derivatives of amino acids on the rectal and intestinal insulin absorption was reported. The present paper described the use of aprotinin as a protecting agent against insulin deactivation in the rectum after rectal administration.

Method

In a microenema dosage form prepared with distilled water, insulin (Crystalline Porcine Insulin, Lilly) was administered to male Sprague-Dawley rats, 200–251 g (fasted 16 h with water available). During the experiments, rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}) and kept on a 38 °C surface. A microenema volume of 1·0 ml kg^{-1} was delivered to the rectum directly via a polyethylene cannula (PE 50). Blood samples were taken from the jugular vein at designated times. Plasma concentrations of glucose were measured by the *o*-toluidine method and plasma insulin levels were measured by a radioimmunoassay (Nishihata et al 1978).

Results

The plasma glucose level after administration of a 1·0 ml kg^{-1} microenema containing 1·0 i.u. of insulin ml^{-1} and 45 mg sodium salicylate ml^{-1} was significantly decreased (Fig. 1). A maximum plasma insulin level of 83·3 ± 20·8 i.u. ml^{-1} (n = 6) was obtained 20 min after microenema administration.

The addition of aprotinin (15 μg ml^{-1} kg^{-1}) to the microenema caused a greater decrease in the plasma glucose level (Fig. 1). The plasma insulin concentration reached a maximum level of 187·4 ± 47·6 i.u. ml^{-1} (n = 6) 20 min after the administration. Aprotinin in an insulin microenema without sodium salicylate caused neither a decrease in plasma glucose levels (normal range indicated by shaded area in Fig. 1) nor an increase in plasma insulin levels which remained below 10 μ i.u. ml^{-1}. This finding indicates that aprotinin does

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not enhance rectal insulin absorption, but may inhibit the degradation of insulin in the rectal area before salicylate-enhanced absorption of insulin.

After an i.v. injection of aprotinin given 5 min before an insulin microenema containing sodium salicylate, no significant difference in the glucose (Fig. 1) or insulin (96·8 ± 26·3 μ i.u. ml^{-1} at 20 min, n = 6) plasma levels could be seen from those obtained without aprotinin pretreatment up to 1 h after microenema administration. After 1 h, no significant difference could be seen between the insulin levels obtained with or without aprotinin pretreatment (24·8 ± 8·6 μ i.u. ml^{-1} and 31·5 ± 10·3 μ i.u. ml^{-1}, respectively, n = 6). However, recovery of the glucose level appeared to be faster with pretreatment than when no pretreatment was given.

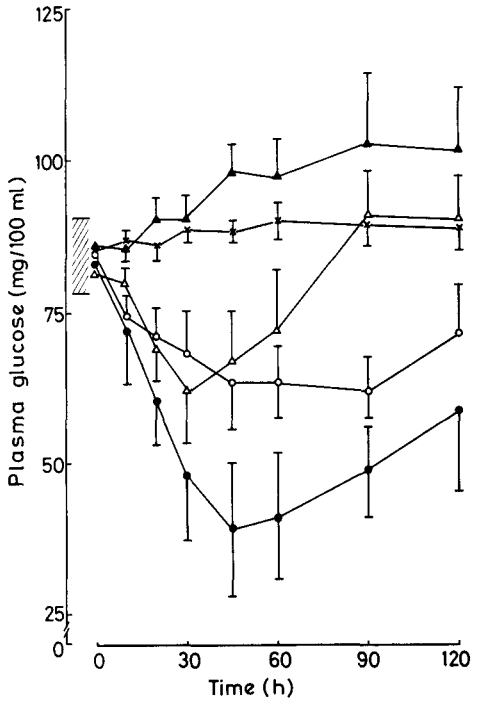

FIG. 1. Plasma glucose levels as a function of time after the rectal administration of a microenema (1 ml kg^{-1}) containing 1·0 i.u. insulin ml^{-1} (—×—); 1·0 i.u. insulin ml^{-1} and 45 mg sodium salicylate ml^{-1} (—○—); 1·0 i.u. insulin ml^{-1}, 45 mg sodium salicylate ml^{-1} and 15 μg aprotinin ml^{-1} (—●—); 1·0 i.u. insulin ml^{-1} and 45 mg sodium salicylate ml^{-1} administered 5 min after an i.v. injection of aprotinin kg^{-1} (—△—). The closed triangle (—▲—) shows the plasma glucose levels obtained after only an i.v. injection of aprotinin (15 μg ml^{-1} kg^{-1}) without any subsequent microenema administration. The shaded area indicates the normal glucose level. The error bars represent the standard deviation with n = to more than 8 ($P < 0·001$ Student's *t*-test for all points, ● vs × or ▲, and ● vs ○ at 30 min; ● vs △ at 45 min; ○ vs △ at 1·5 h; ○ vs × or ▲, and △ vs × or ▲ during the first 60 min).

To further examine the effect of aprotinin on the plasma glucose level, an i.v. injection of 15 μg ml^{-1} kg^{-1} aprotinin was given without insulin rectal administration. A slight increase in the plasma glucose level was seen (Fig. 1). However, when an aprotinin injection of lower concentration (2·0 μg ml^{-1} kg^{-1}) was administered, the plasma glucose level did not appear to increase. Therefore, we speculate that a high concentration of aprotinin in the plasma may affect the plasma glucose level. This finding, however, differs from an earlier report (Berger et al 1980) which states that aprotinin does not affect glucose plasma levels.

When salicylate and 15 μg ml^{-1} kg^{-1} aprotinin were rectally administered together, the change in the plasma glucose level was not significant since the glucose level remained within the normal glucose range. However, the administration of aprotinin in conjunction with both insulin and promoting agents of insulin rectal absorption appears to afford a more effective pharmaceutical approach for the rectal delivery of insulin into the body.

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