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Poly(alkyl cyanoacrylate) Nanocapsules as a Delivery System in the Rat for Octreotide, a Long-acting Somatostatin Analogue

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Abstract

Poly(alkyl cyanoacrylate) nanocapsules have been used as biodegradable polymeric drug carriers for subcutaneous and peroral delivery of octreotide, a long-acting somatostatin analogue; their ability to reduce insulin secretion or prolactin secretion in response to oestrogens has been studied in adult male rats.

The nanocapsules, prepared by interfacial emulsion polymerization of isobutyl cyanoacrylate, were 260 nm in diameter and incorporated 60% of octreotide. Administered subcutaneously, the octreotide-loaded (20 $\mu g\ kg^{-1}$) nanocapsules suppressed the insulinaemia peak induced by intravenous glucose overload and depressed insulin secretion over 48 h, preventing the secretory rebound; however, glycaemia was unaffected. In parallel, the plasma octreotide concentration increased 2·7 times. Administered perorally to oestrogen-treated rats, octreotide-loaded nanocapsules (200 and 1000 $\mu g\ kg^{-1}$) significantly improved the reduction of prolactin secretion (by 72 and 88%, respectively, compared with 32 and 54% with free octreotide) and slightly increased plasma octreotide level.

Thus nanocapsules could be of interest as a biodegradable drug carrier for the administration of octreotide.

Somatostatin (SRIF-14) is a naturally occurring tetradecapeptide expressed by the hypothalamus and the gastrointestinal tract complex (pancreas, visceral anatomic nervous system, endocrine cells, gut lumen). It exerts pluripotent biological actions. Besides its central growth-hormone release-inhibiting effect, it depresses many endocrine and exocrine secretions (insulin, glucagon, pancreatic polypeptide, pancreatic enzyme and bicarbonate responses to cholecystokinin and secretin) and reduces gastrointestinal motility and blood flow (Reichlin 1983a, b; Harris 1994). However, its short half-life of 2–3 min necessitates its application by intravenous infusion, thus limiting its therapeutic use. Thus more potent longer-acting analogues have been developed to overcome the limitations of native somatostatin. One is octreotide (SMS 201-995, Sandostatin; Novartis Pharma, Basle, Switzerland), a synthetic octapeptide which retains the essential pharmacophore portion (Phe-Trp-Lys-Thr) of the native molecule (Veber et al 1979, 1981). It has greater pharmacological activity than SRIF-14, being 70, 23 and 3 times more potent at inhibiting growth hormone, glucagon and insulin secretion, respectively (Bauer et al 1982; Pless et al 1986). It also has a longer duration of action, with a half-life of 113 min (Marbach et al 1985). Thus, octreotide has been used, in man, for the treatment of acromegaly (Vance et al 1991; Andersen et al 1995), Zollinger Ellison syndrome, non-tumoural secretory diarrhoea (Rosenberg 1988), insulin-dependent diabetes (Candrina & Giustina 1988; Lunetta et al 1996; Orskov et al 1996) and secreting and non-secreting tumours (Rosenberg 1988; Weckbecker et al 1992, 1993; Arnold et al 1996; Robbins 1996). Finally, octreotide is more stable than SRIF-14 and can be given parenterally by subcutaneous injection. It can also be administered orally, but with low bioavailability (Williams et al 1986a).

Nanocapsules composed of a biocompatible and biodegradable polymer, prepared by interfacial polymerization of alkyl cyanoacrylate (Al Khouri et al 1986), have been developed as vesicular colloidal polymeric drug carriers to improve the therapeutic efficacy of drugs. Poly(alkyl cyanoacrylate) drug carriers have been shown to improve the biological action of indomethacin, alvarol, vincamine and insulin after subcutaneous and peroral delivery (Maincent et al 1986; Damgé et al 1988, 1990, 1995; Ammoury et al 1991; Beck et al 1994). In fact, nanocapsules protected the peptides against proteolytic degradation in the gastrointestinal tract (Damgé et al 1990, 1995; Ammoury et al 1991; Michel et al 1991; Lowe & Temple 1994) and transported them across the intestinal mucosa via a paracellular pathway (Aprahamian et al 1987; Damgé et al 1990). Thus it was of interest to determine whether nanocapsules could also improve the biological response of other peptides, for example octreotide. We have prepared octreotide–poly(isobutyl cyanoacrylate) nanocapsules and analysed two biological effects, the insulin response to glucose and the inhibition of prolactin secretion in response to oestrogens of the peptide, after subcutaneous and peroral administration in the rat. Plasma levels of octreotide were also measured under these experimental conditions.

Materials and Methods

Preparation and characterization of nanocapsules

Nanocapsules were prepared by interfacial emulsion polymerization of isobutyl cyanoacrylate by the method of Al Khouri et al (1986). Octreotide (Novartis Pharma, Basle, Switzerland) was added to a lipophilic phase containing miglyol (Dyna-France, Paris, France; 1 mL) and isobutyl 2-cyanoacrylate (Ethnor, Paris, France; 0·125 mL) dissolved in absolute ethanol (25 mL). After addition of this phase to an aqueous solution (50 mL) of a non-ionic surfactant (Poloxamer 188, ICI, Clamart, France; 0·25%), nanocapsules were immediately formed under the action of mechanical stirring. The

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suspension obtained was then concentrated to a final volume of 12·5 mL by evaporation under vacuum. The final suspension of nanocapsules contained 200 μg octreotide mL^{-1} and the efficiency of encapsulation was approximately 60%.

For control experiments, empty nanocapsules were prepared by the same procedure but without addition of octreotide. An emulsion of octreotide was also prepared by the same technique but without addition of isobutyl cyanoacrylate.

The size of nanocapsules, estimated by a laser-light scattering method with a monochromatic laser ray diffusion counter (Autosizer 2C, Malvern Instruments, Les Ulis, France), was, respectively, 250 and 260 nm for empty and octreotide-loaded nanocapsules.

Experimental procedures

Octreotide incorporated or not in poly(alkyl cyanoacrylate) nanocapsules was administered either subcutaneously or intragastrically by force-feeding of adult male Wistar rats.

Subcutaneous administration of octreotide-loaded nanocapsules

Twenty adult rats, 300–350 g, were fasted overnight. They were divided into 4 groups each of 5 rats and subjected to a single subcutaneous injection of either octreotide-loaded nanocapsules (20 $\mu g\ kg^{-1}$) or octreotide in an emulsion (20 $\mu g\ kg^{-1}$). Control animals were subjected to subcutaneous injection of empty nanocapsules or normal saline. All injections were performed in a volume of 1 mL kg^{-1}. Thirty minutes later all rats received an intravenous injection of 10% glucose (0·15 g kg^{-1} body weight). In all groups blood was collected from the tail just before the subcutaneous injections and the intravenous glucose injections and 30 min and 2, 6, 24 and 48 h after the injection of intravenous glucose, for analysis of plasma levels of glucose, insulin and octreotide.

Peroral administration of octreotide-loaded nanocapsules

The biological effect of octreotide-loaded nanocapsules was analysed by measurement of the inhibition of the secretion of prolactin induced by oestrogens.

Twenty-four adult male Wistar rats, approximately 150 g, were implanted subcutaneously with an oestradiol (50 mg β-oestradiol-3-benzoate)-containing silastic tube closed at both sides with silicone rubber (CAF 4 Rhodorsil silicone, Rhône Poulenc, Saint Fons, France) for at least a month to elevate prolactin secretion artificially; under these conditions this is susceptible to inhibition by somatostatin and its analogues. These animals were divided into four groups of six rats and fasted overnight. Octreotide incorporated or not in nanocapsules was given intragastrically by gavage in a single administration of either 200 or 1000 $\mu g\ kg^{-1}$. Blood was sampled from the tail vein just before gavage and 30 min, 2 and 4 h after for the first day, then every three days until 21 days to determine levels of prolactin and octreotide.

Biochemical and radiochemical assays

Plasma-glucose concentrations were measured by means of the glucose oxidase method (Huggett & Nixon 1957).

Radioimmunological methods were used to measure insulin, octreotide and prolactin levels in the blood. Blood samples were collected in chilled tubes containing 20 μL aprotinin (zymofren) and 15 μL heparin. Plasma was separated by 5 min centrifugation at 10000 g and 4°C. The plasma was kept frozen until analysis.

Plasma immunoreactive insulin was measured by the method of Herbert et al (1965) using the anti-insulin antibody INSIK from the CEA (Commissariat à l'Energie Atomique, France). The analytical sensitivity, defined as the concentration of insulin displacing 5% of the initially bound tracer, was 0·1 ng mL^{-1}.

Plasma immunoreactive octreotide and prolactin were assayed according to the technique described by Marbach et al (1985). All samples were determined in duplicate; the detection limit was approximately 10 pg mL^{-1} for octreotide and 0.2 ng ml^{-1} for prolactin.

Statistical analysis

The means and standard errors of the means were calculated for all values. One-way analysis of variance followed by a Neumann-Keuls test was used for group comparisons.

Results

Effects of subcutaneous administration of octreotide and of octreotide-loaded nanocapsules on plasma glucose and insulin concentrations in the rat

In rats, intravenous glucose injection (0·15 g kg^{-1}) provoked an immediate increase in glycaemia. Glucose level increments reached 161 ± 6 mg dL^{-1} within 30 min (Fig. 1). Glycaemia then progressively returned to basal values which were recovered within 2 h. The subcutaneous administration of 20 $\mu g\ kg^{-1}$ octreotide or octreotide-loaded nanocapsules, 30 min before the glucose tolerance test, did not change these profiles significantly. Empty nanocapsules were also without any effect on the glycaemic profile (Fig. 1).

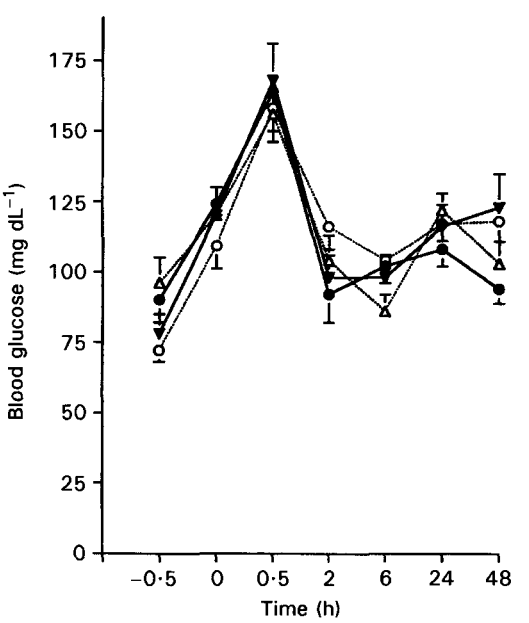

FIG. 1. Glycaemia of intravenous overload of glucose (0·15 g kg^{-1}) given 30 min after subcutaneous administration of 20 $\mu g\ kg^{-1}$ octreotide either incorporated in nanocapsules (○) or as an emulsion (●). Control animals received empty nanocapsules (△) or physiological saline (▲). Data are means ± s.e.m. of results from five animals.

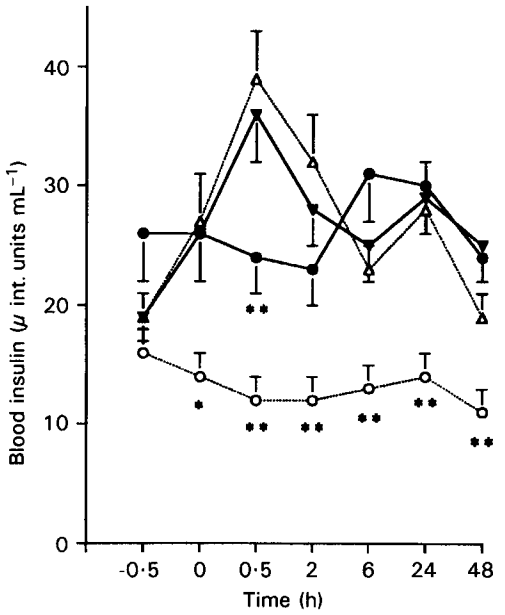

FIG. 2. Insulinaemia after intravenous overload of glucose (0·15 g kg^{-1}) given 30 min after subcutaneous administration of 20 μg kg^{-1} octreotide either incorporated in nanocapsules (○) or as an emulsion (●). Control animals received empty nanocapsules (△) or physiological saline saline (▲). Data are means ± s.e.m. of results from five animals. $*P < 0.05$, $**P < 0.01$ compared with controls.

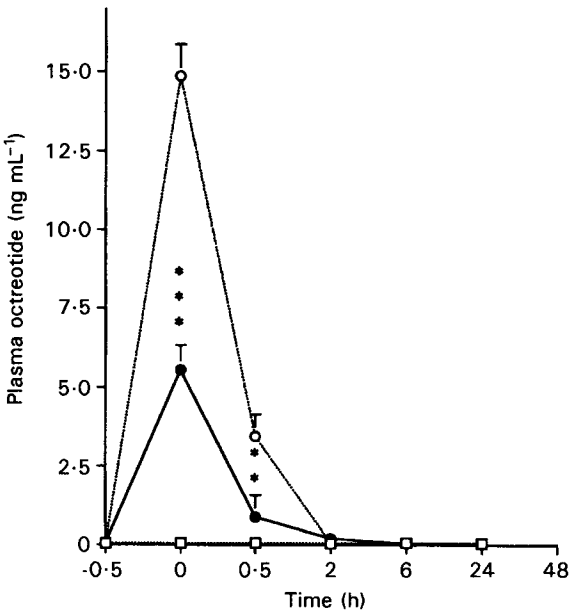

FIG. 3. Plasma octreotide concentration of subcutaneous administration of 20 μg kg^{-1} octreotide either incorporated in nanocapsules (○) or not (●). Control animals received subcutaneous administration of empty nanocapsules (□). Data are means ± s.e.m. of results from five animals. $**P < 0.01$, $***P < 0.001$ compared with controls.

As depicted in Fig. 2, the intravenous glucose overload was also accompanied by an increase in plasma insulin concentrations which peaked 30 min after administration of glucose. Then insulinaemia decreased progressively, attaining normal values from 2 h afterwards.

Empty nanocapsules, administered subcutaneously 30 min before glucose injection, did not modify this profile.

However, the subcutaneous administration of 20 μg kg^{-1} octreotide markedly reduced insulinaemia over a period of 2 h. Indeed, not only did the peak of insulinaemia not appear but plasma insulin concentration was 11% lower than basal values. After 6 h, a slight increase of insulinaemia (+22%) was observed but basal values were again reached after 48 h.

Octreotide-loaded nanocapsules (20 μg kg^{-1}) reduced insulinaemia more than non-encapsulated octreotide. Thirty minutes after subcutaneous injection, insulinaemia was already reduced by 14% ($P < 0.05$). More interesting was the complete suppression of the peak of insulinaemia 30 min after glucose overload, followed by a sustained depression of insulin secretion over at least 48 h. Indeed, all values were much lower than normal values (−45%, $P < 0.01$). The peak of insulinaemia was reduced by 70%, compared with 35% for non-encapsulated octreotide.

The plasma level of octreotide is depicted in Fig. 3. After subcutaneous administration of 20 μg kg^{-1} octreotide, this parameter increased rapidly attaining a peak after 30 min. Control values were restored after 6 h.

Octreotide nanocapsules administered subcutaneously at the same concentration significantly ($P < 0.001$) increased this peak by 74% at 30 min. The plasma octreotide concentration remained elevated for 2 h (+65%, $P < 0.01$) but control values were again restored after 6 h.

Thus, the incorporation of octreotide in nanocapsules improved and markedly prolonged its biological action after subcutaneous administration.

Effects of a peroral administration of octreotide and octreotide-loaded nanocapsules on plasma prolactin concentrations induced by oestrogens in the rat

As depicted in Fig. 4, octreotide (200 μg kg^{-1}), administered by gavage to rats subjected to oestrogen implants, markedly reduced prolactin secretion after 30 min (−30%, $P < 0.05$), the maximum reduction being observed after 2 h (−32%, $P < 0.01$). A secretory rebound appeared after 4 h but the plasma prolactin concentration remained low (−24%) from day 3 to day 12 and increased again progressively, reaching control values from day 15.

When octreotide (200 μg kg^{-1}) was incorporated in nanocapsules, the reduction of plasma prolactin concentration was greater after 30 min (−72%, $P < 0.001$) (Fig. 4). The concentration of prolactin was still low after 2 h (−50%, $P < 0.01$)) and the secretory rebound also appeared after 4 h. Then, from day 3 to day 21, the plasma prolactin concentration remained lower (−40 to −50%) than with non-encapsulated octreotide treatment.

When a higher concentration of octreotide (1000 μg kg^{-1}) was administered perorally to rats, the reduction of plasma prolactin concentration was greater (−54%, $P < 0.01$%, at 30 min and −24% at 2 h) than with 200 μg kg^{-1} octreotide (Fig. 5). Then a slight secretory rebound was observed up to the end of the experiment.

The early reduction of plasma prolactin concentration was more intense (−88% at 30 min, $P < 0.001$, and −58% at 2 h, $P < 0.01$) when octreotide (1000 μg kg^{-1}) was incorporated in

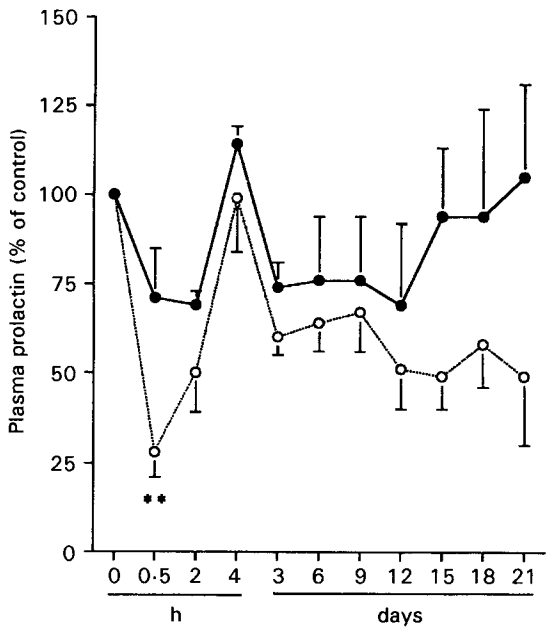

FIG. 4. Prolactin plasma concentration after single intragastric dose of octreotide (200 μg kg^{-1}) incorporated in nanocapsules (○) or not (●) in oestrogen-treated animals. Data, expressed as percentages of control values obtained just before octreotide administration, are means ± s.e.m. of results from six animals. $^{**}P < 0.01$ compared with controls.

nanocapsules (Fig. 5). However, thereafter there was no significant difference between the profiles.

The plasma octreotide concentrations after peroral administration of octreotide (200 and 1000 μg kg^{-1}) incorporated or not in nanocapsules are depicted in Fig. 6. In all cases, plasma octreotide concentrations peaked 30 min after gavage. This peak was higher with octreotide 1000 μg kg^{-1} than with octreotide 200 μg kg^{-1} and slightly more elevated with encapsulated octreotide than with non-encapsulated drug.

Thus the incorporation of octreotide in nanocapsules improved the biological action of octreotide administered perorally to rats, in a dose-dependent manner.

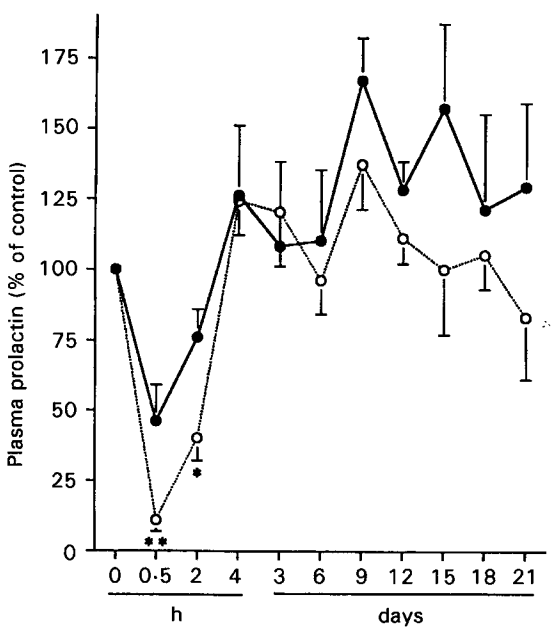

FIG. 5. Prolactin plasma concentration after a single intragastric dose of octreotide (1000 μg kg^{-1}) incorporated in nanocapsules (○) or not (●) in oestrogen-treated animals. Results, expressed as percentages of control values obtained just before octreotide administration, are means ± s.e.m. of results from six animals. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with controls.

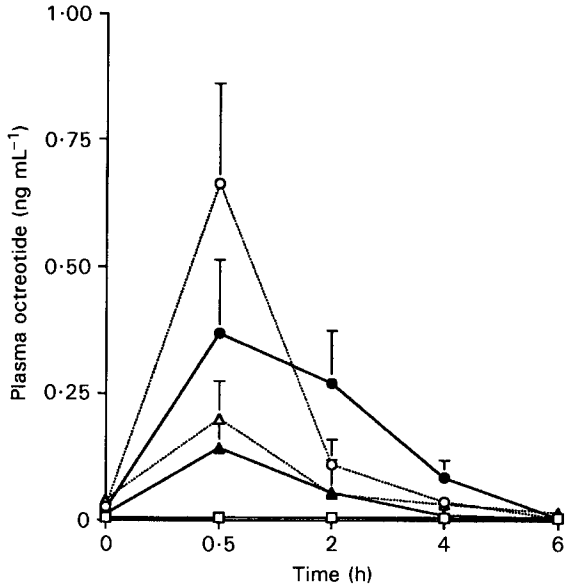

FIG. 6. Octreotide plasma concentration after a single intragastric dose of octreotide (—) and octreotide incorporated in nanocapsules (-----). Two concentrations of octreotide were used, 200 μg kg^{-1} (△ and ▲) and 1000 μg kg^{-1} (○ and ●). Control animals received a subcutaneous dose of empty nanocapsules (□). Data (ng mL^{-1}) are means ± s.e.m. of results from six animals.

Discussion

This work clearly indicates that the incorporation of octreotide in poly(isobutyl cyanoacrylate) nanocapsules improved and prolonged the therapeutic effect of subcutaneously administered octreotide, as measured by the secretion of insulin in response to glucose overload. Indeed, whereas non-encapsulated octreotide only suppressed the insulinaemia peak which appeared 30 min after intravenous injection of glucose, octreotide incorporated in nanocapsules completely depressed the secretion of insulin for a prolonged period which certainly exceeded 48 h, the duration of our experiment. We have previously reported similar results for the incorporation of another peptide, insulin, in nanocapsules (Damgé et al 1988). Indeed, after subcutaneous administration of insulin-loaded nanocapsules in both diabetic and non-diabetic rats, glycaemia decreased for 20 to 24 h, compared with 4 h with free insulin. Thus incorporation of the peptide in the colloidal polymeric drug carrier considerably prolonged its biological activity.

With octreotide the interesting feature was that its incorporation in poly(alkyl cyanoacrylate) nanocapsules also inhibited the secretory rebound of insulin which appeared between 6 and 28 h with free octreotide. The secretory rebound, already described by others (Del Pozzo et al 1986;

Lembcke et al 1987) was attributed to longer suppression of GH secretion than insulin secretion.

The inhibition of the peak of insulinaemia in response to a glucose overload could be explained by the release of unencapsulated octreotide, because only 60% was incorporated in the nanocapsules, and by the liberation of octreotide by the nanocapsules themselves. Indeed, the plasma octreotide level was higher than that noted after treatment with free octreotide. The sustained depression of insulinaemia over at least 48 h could be attributed to progressive release of octreotide by degradation or bioerosion of the polymer. It is well known that poly(alkyl cyanoacrylate) nanocapsules can be degraded under physiological conditions. The most probable means of degradation might be enzymatic hydrolysis of the polymeric side ester chains leading to dissolution of the polymer (Lenaerts et al 1984). Esterases might be mainly involved in this phenomenon (Scherer et al 1994) leading to the production of poly(cyanoacrylic acid) and alcohol (Lenaerts et al 1984). The duration of this means of degradation was estimated as more than 24 h after subcutaneous administration of labelled nanoparticles (Grislain et al 1983).

Generally, glucose overload induces a simultaneous peak in glycaemia and insulinaemia. We also observed this phenomenon; glycaemia increased 30 min after intravenous injection of glucose, then returned to normal values from 2 h. Surprisingly, octreotide, whether encapsulated or not, did not alter this profile. In the healthy man the subcutaneous administration of octreotide was followed by an increase of glycaemia observed 1 or 2 h after a meal, the intensity of this increase depending on the composition of the meal and the octreotide dose (Williams et al 1986b; Lembcke et al 1987). In fact, glycaemia is regulated by three hormones: growth hormone and glucagon leading to hyperglycaemia, and insulin leading to hypoglycaemia. Thus the discrepancies between man and rat might be explained, in the rat, by greater selectivity of octreotide for glucagon than for insulin. In man it seems that this selectivity does not exist (Williams et al 1986b).

This work also indicates that the incorporation of octreotide in nanocapsules was able to improve its biological action after peroral administration in the rat. The parameter which was tested was the secretion of prolactin in response to oestrogens, this parameter being less sensitive to stress. Octreotide, in contrast with other peptides, is more stable against proteolytic enzymes and can be absorbed to a variable extent in the different segments of the small intestine. Direct absorption via enterocytes was demonstrated preferentially in the jejunum (Fricker et al 1991), leading to significantly elevated octreotide plasma concentrations that are sufficient to suppress GH secretion (Köhler et al 1987). These results agree well with our current findings; after peroral administration of octreotide in the rat, the plasma concentration of octreotide increased in parallel with the octreotide dose. At the same time the prolactin secretion stimulated by oestrogens decreased, maximum responses being noted 30 min after gavage. The incorporation of octreotide in nanocapsules increased this effect, both on prolactin secretion and on plasma octreotide concentration. This rapid effect could be attributed to the absorption of octreotide liberated in the intestinal lumen by nanocapsules (Beck et al 1994; Lowe & Temple 1994) and by the absorption of non-encapsulated octreotide present in the preparation. The presence in the preparation of surfactants and miglyol, considered to be absorption enhancers, could facilitate the absorption of octreotide (Masuda et al 1986). However, direct transport of octreotide-loaded nanocapsules from the intestinal lumen to the blood compartment cannot be excluded, though the intensity of this mechanism is difficult to determine. Indeed, nanocapsules have been found to cross the intestinal mucosa all along the small intestine using a paracellular pathway (Aprahamian et al 1987; Damgé et al 1990; Michel et al 1991); however, their passage was more pronounced in the ileum, via the numerous lymph nodes (Damgé et al 1990). Whatever the mechanism of absorption of octreotide, it is not easy to understand why octreotide nanocapsules were efficient for only 2 h after a peroral administration, whereas insulin nanocapsules were biologically active for several weeks (Damgé et al 1988, 1990, 1995). In fact, the interactions between isobutyl cyanoacrylate and octreotide or insulin are probably different, insulin playing the role of a polymerization initiator.

In conclusion, poly(alkyl cyanoacrylate) nanocapsules can be considered a suitable carrier for octreotide. They are biodegradable and considerably prolonged the biological activity of octreotide after subcutaneous administration, preventing adverse effects, namely the secretory rebound. They also improve the biological response to the drug after peroral administration.

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