A New Route of Drug Administration: Intrauterine Delivery of Insulin and Calcitonin

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High molecular weight drugs in general, and peptides in particular, are usually delivered by parenteral route because they are poorly absorbed or degraded in the gastrointestinal tract. To optimize therapy, it is desirable to search for nonparenteral routes of administration and to deliver the drug in a controlled-release fashion. We report here on the absorption and the systemic biological effect of two peptides, insulin and calcitonin, after instillation into the uterus of the rat. Intrauterine delivery was compared to subcutaneous injections in intact and ovariectomized rats. In addition, we describe results of a preliminary study on calcitonin absorption from controlled-release matrices inserted in the rat uterus. The amount and duration of the hypoglycemic and the hypocalcemic effects induced by intrauterine delivery of insulin and calcitonin, respectively, were equivalent to those obtained after subcutaneous injections. The results were similar in intact and ovariectomized rats. It is concluded that the intrauterine administration of both insulin and calcitonin is bioequivalent to subcutaneous injection. The therapy of a number of clinically important diseases could benefit from this discovery.

KEY WORDS: drug administration; drug delivery; absorption; controlled release; drug implants; peptides; intrauterine; calcitonin; insulin.

INTRODUCTION

Clinically, the most common and accepted way for delivering drugs having relatively low molecular weights (MW; 400 to 600 Da), is the oral route. High MW drugs and very polar drugs, in general, and peptides/proteins, in particular, are usually delivered by parenteral routes because they are poorly absorbed (low permeability) or extensively degraded (metabolized) in the gastrointestinal tract. Long-term, repeated injections are often required because of the drug's short half-life and the chronic nature of many diseases. Parenteral therapy is inconvenient, requires medical supervision, and may result in poor patient compliance. To optimize therapy, it is desirable to search for nonparenteral routes of drug administration and, for many applications, to deliver the drug in a controlled-release (i.e., continuous or pulsatile) fashion.

In order to overcome some of the drawbacks associated with parenteral administration, considerable attention has been focused on the development of transmucosal, rectal, nasal, buccal, ophthalmic, transdermal, and vaginal routes of administration (1,2). However, each of the routes mentioned above has inherent limitations such as proteolytic inactivation, low tissue permeability, and poor drug stability. Furthermore, some routes are poorly tolerated, inconvenient, and limited in application to a small number of drugs. For example, vaginal administration has been extensively studied for systemic drug administration (3). However, the vaginal bioavailability of peptides and proteins is low and too variable to be useful clinically (3). There is, therefore, a need for the development of new routes of drug administration for drugs with low oral bioavailability, such as peptide/proteins drugs.

We propose here a new route for drug administration, i.e., intrauterine drug delivery. We discovered (4) that high MW drugs such as insulin (6000 Da) and calcitonin (3450 Da) are absorbed in biologically active form from the uterus of rats. Our method may be suitable for postmenopausal women without menses. In this paper we describe the absorption and the systemic biological effect of two peptides, insulin and calcitonin, from uterus of rats. Intrauterine (iu) delivery was compared to subcutaneous (sc) injections in intact and ovariectomized rats. In addition, we describe here insulin and calcitonin absorption and biological effects following iu and sc implantation of controlled-release devices.

MATERIALS AND METHODS

Materials

Human insulin (Actrapid HM, Novo, Bagavaerd, Denmark), salmon calcitonin (Micalcic, Sandoz, Switzerland), and human calcitonin (Ciba-Geigy, UK) were used. Nonradiolabeled and ^{14}C-radiolabeled disodium hydroxyethylidene bisphosphonate (HEBP; sp act 48.9 mCi/mol) were obtained from Prof. R. J. Levy (5). Pellethane 80 AE (Dow Chemicals) was used for the preparation of drug delivery matrices. All other materials were of analytical grade.

In Vitro Diffusion Studies

The permeability of the rat's uterus to model drugs was studied *in vitro* using excised uteri of intact and ovariectomized rats (30 days postovariectomy; see below). A 100-μL solution of the model drug was instilled in the lumen of each uterine horn, by a blunted 21-G, 1.5-in. syringe inserted through the vagina of the excised uterus. The drugs examined were insulin (200 μU/mL), methylene blue hydrochloride (10%, w/v), and HEBP (20%, w/v). Methylene blue and HEBP were selected as model drugs since they are positively and negatively charged molecules, respectively, at physiologic pH. In addition, the tissue staining by methylene blue facilitates easy detection of the drug. The cervix was ligated with surgical suture and each uterus was immersed in 100 mL of Kreb's Ringer buffer (bubbled for 10 min with 95% oxygen/5% CO_2). The buffer solution was stirred with a magnetic stirrer and was sampled (50 μL) at specified time points (n = 8 in each group). Insulin concentration was determined by the RIA kit (see below), and methylene blue by UV/vis spectrophotometry. HEBP concentration was deter-

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mined by combining 50 μL and 2.5 mL of scintillation fluid (Atom light, NEN, Boston, MA) and counting the mixture for carbon-14 activity in a Beckman LS 3801 liquid scintillation counter (Beckman Instruments, Berkely, CA), with reference to a calibration curve. Counting efficiency was determined from a series of quenched Beckman standards and identical control solutions.

Intrauterine Delivery—Surgical Procedures

For the iu delivery studies, a blunted syringe (21 G, 1.5 in.) was inserted through the vagina of ether-anesthesized rats into the uterus. The exact location of the needle tip in the uterus lumen was monitored through an abdominal incision. Special care was taken not to injure the uterus. Drug (HEBP, insulin, or calcitonin; see below) or saline was instilled as equally divided volumes (0.1 mL) into the two horns of each uterus. The syringe was removed and the abdominal incision was closed with surgical staples.

In some experiments [0.4 U/kg insulin study and in 10 animals (sc and iu) in the 0.8 U/kg calcitonin study], the cervix was ligated with surgical sutures in order to assure no leaks of the administered solution to the vaginal region. In another experiment (0.8 U/kg calcitonin), the uterus was ligated at the cervix and at the distal end of the uterine horns (in the oviduct/uterus region) in order to assure no leaks of the administered solution to the oviducts/ovaries/peritoneum. No ligations of the uterus were performed in further experiments since no differences in serum drug concentrations or biological effects were observed between ligated and nonligated animals. Blood samples were collected from the tail artery under mild anesthesia at specified time points and analyzed for insulin, glucose, calcium, and calcitonin (see below). In most of the insulin delivery studies the rats were fasted overnight prior to sampling (see below). In order to avoid chronobiological effects, treatments and sampling were performed during the same time period each day (morning).

Insulin Administration

Female Sabra rats (Faculty of Medicine, The Hebrew University of Jerusalem, Israel) weighing approximately 250 g were used. For iu absorption studies with insulin, three experimental groups were used: (i) iu administration of insulin, (ii) iu administration of saline, and (iii) sc injections of insulin. A solution (0.2 mL) containing insulin (0.4 and 4 U/kg body weight) was instilled in equally divided volumes into the two horns of the uterus. Saline was instilled instead of insulin in the control group. The positive control group received the same dose of insulin as above subcutaneously. Sampling for serum insulin and glucose was performed at specified time points as described above. In the 0.4 U/kg dose study, the rats were fasted over night. Serum glucose and insulin levels were measured by glucose oxidase determination and radioimmunoassay (Sorin, Italy), respectively (6).

Controlled-Release Administration of Insulin in Diabetic Rats

Experimental diabetes was induced in female rats (n = 17; 200 g) by daily iv injections of streptozocin solution for 3 days (freshly prepared to avoid decomposition, 50 mg/kg in 0.01 M sodium citrate buffer, pH 4.5). Five days later the biological effect of controlled-release insulin was studied by inserting a sustained-release insulin implant (Linplant, Linshin, Scarborough, Canada) into the uterus using a 12-G blunted needle by the same procedure as above. These pellets release 2 U/day, and the iu delivery was compared to that in rats with subdermal implants. The control group (untreated) was sham-operated. Blood sampling was performed, as detailed above, following an overnight fast.

Calcitonin Administration

Intact (same as above) and ovariectomized rats were used in this study. Ovariectomized rats were used in order to examine drug absorption from the atrophied uterus (7). The ovaries of female rats weighing ~200 g were excised under ether anesthesia ("ovariectomized"). One month postoperation the ovariectomized and intact animals' groups were randomly divided into three subgroups and treated as follows: (i) sc injections of salmon calcitonin, (ii) iu administration of salmon calcitonin, and (iii) iu administration of saline. The salmon calcitonin dose in both iu and sc groups was 0.8 U/kg body weight (0.2 mL). The procedures for the iu administration of salmon calcitonin solution and blood collection were the same as detailed in the insulin delivery experiments. Serum calcium concentration was determined by atomic absorption spectroscopy (8).

Controlled-Release Administration of Calcitonin

Two types of drug delivery devices were examined: mini osmotic pumps (ALZET 2001, Alza Corp., Palo Alto, CA), and polyurethane matrices (9,10). The mini osmotic pumps were filled with human calcitonin solution delivering 1 U/kg/day for 14 days. Polyurethane (Pellethane 80AE, Dow Chemicals) matrices containing 2% (w/w) human calcitonin and 15% (w/w) polyethylene glycol 3000 were casted on glass petri dishes from a 10% (w/v) solution in dimethylacetamide as described previously (9,10).

A small incision was made just above the cervix in the right horn of anesthesized ovariectomized rats (see above). Anesthesia was induced by intraperitoneal injection of 0.25 mL/100 g body weight of a solution containing 12.75 g chloral hydrate, 3.6 g pentobarbital, 6.5 g $MgSO_4$, 23 mL ethanol (95%), 108 mL double-distilled water, and 108 mL propylene glycol. A matrix 1.5 × 1 cm and 0.156 ± mm thick was rolled into a cylinder shape and was inserted into the right horn of the uterus. The wound was closed with medical glue (Histoacryl blau, B. Braun, Melsungen, Germany). The control group consisted of sham-operated animals. At specified time points serum human calcitonin levels were measured with a radioimmunoassay kit (ELISA-hCT, CIS Biointernational, France), and the background levels in the sham-operated rats were treated as "blank" for the calibration curve.

For the mini osmotic pumps study, another group of ovariectomized rats was divided into two subgroups: iu and sc administration. In the iu delivery subgroup one mini osmotic pump was implanted subdermally and a plastic cannula connected to the device tip was inserted through the cervix to the uterus. In the second subgroup one device was

implanted in a subdermal pouch for sc administration as described previously (11).

Data Analysis

Data are expressed as the mean ± standard error (SE). The areas under the curves (AUCs) of serum insulin and calcium concentrations for both the iu and the sc routes (0 to 24 hr) were calculated by a computerized linear trapezoidal method (12). The significance of differences between calculated AUCs was assessed with the Mann–Whitney test. Results were termed significant at $P < 0.05$. All calibration curves used in the various analyses (UV/vis, radioactive measurements, and atomic absorption spectroscopy) had a correlation coefficient of at least 0.997.

RESULTS

In Vitro Diffusion Studies

The diffusion of insulin, methylene blue, and HEBP across uterine tissue *in vitro* is depicted in Fig. 1. Similar, rapid transport of insulin across uterine tissue of both intact and ovariectomized rats was observed; after ~2 hr the concentrations in the receiving and the donor solution were equal. In contrast, insignificant concentrations of both methylene blue and HEBP were found in the receiving solution, even after 24 hr.

Intrauterine Delivery of Insulin

Acute Administration

Serum insulin and glucose levels in rats treated with 0.4 U/kg iu insulin are described in Fig. 2. Similar profiles of increased serum insulin concentration were obtained by sc injections and iu delivery. Insulin absorption by both routes resulted in the same significant reduction in serum glucose levels (Fig. 2, bottom). Maximal increases in serum insulin concentration were seen between 0.5 and 2 hr after administration for both the iu and the sc routes. Maximal decreases in glucose concentrations were seen shortly after the appearance of the insulin peak and between 2 and 4 hr following insulin administration. No changes were observed in both insulin and glucose levels in rats treated with iu saline administration. The extent of insulin absorption after iu administration was found to be equivalent to that after sc administration; the differences in the AUCs were negligible (152 ± 16 and 160 ± 16 μU/mL × hr). Similar results were obtained when higher insulin concentrations (4 U/kg) were used (351 ± 76 and 275 ± 38 μU/mL × hr, respectively).

Controlled-Release Administration

After iu and sc administration of insulin by controlled-release pellets in diabetic rats, serum insulin concentrations were found to rise steadily throughout the experiment, to reach four times the basal levels of insulin, and glucose concentrations were found to fall steadily to approximately normal basal levels of glucose (Fig. 3). No changes were observed in insulin or glucose levels in diabetic rats treated with iu saline.

Intrauterine Administration of Calcitonin

Acute Administration

The serum calcium concentrations in intact rats treated with iu calcitonin are described in Fig. 4. Similar profiles of serum calcium levels were obtained following sc and iu ad-

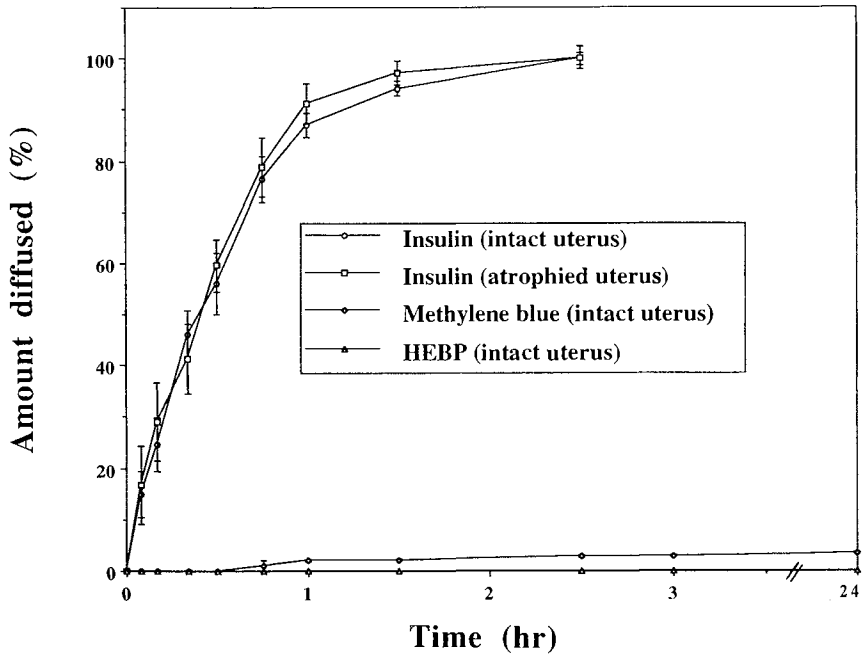

Fig. 1. The diffusion of insulin, methylene blue, and HEBP across uterine tissue *in vitro*. Each point represents the ratio ± SE of the mean concentration in the receiving solution to the initial concentration in the uterine lumen ($n = 8$).

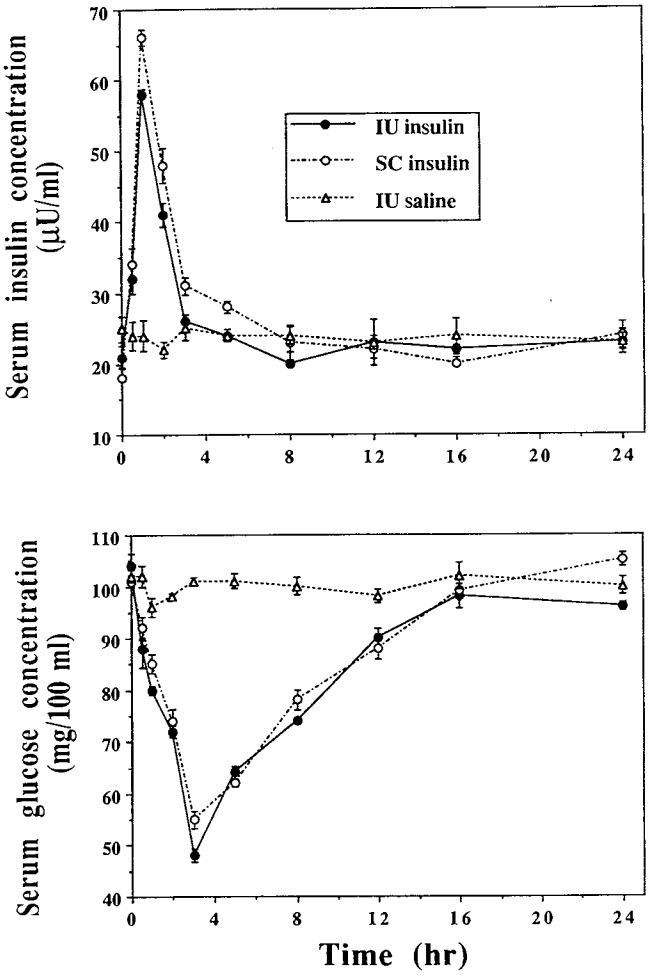

Fig. 2. Serum insulin (top) and glucose (bottom) levels in rats treated with 0.4 U/kg body weight insulin by the iu route and by subcutaneous injections. Each point represents the mean ± SE of five rats.

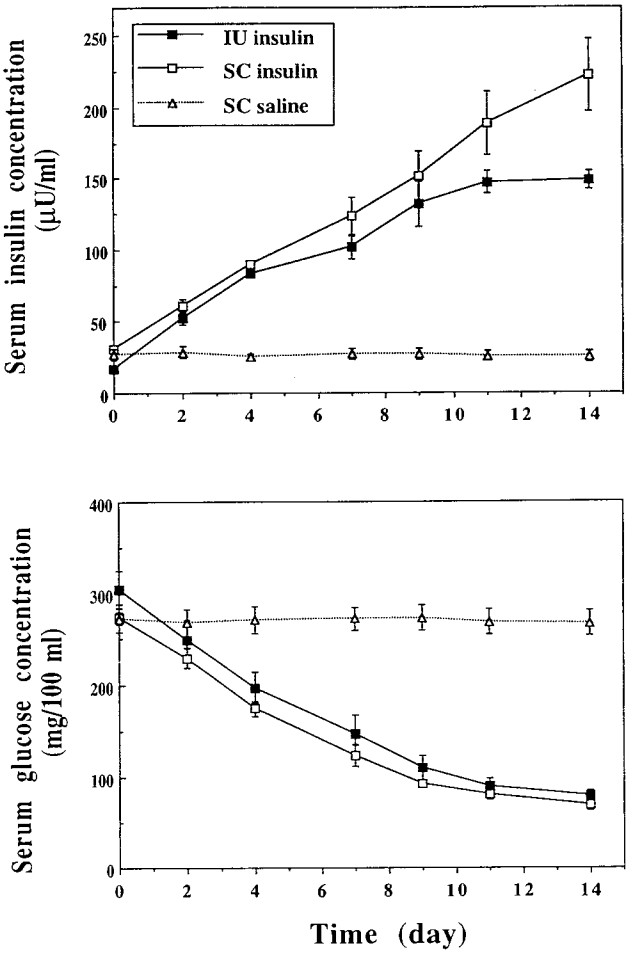

Fig. 3. Serum insulin and glucose levels following iu and sc implantation of insulin pellets. Each point represents the mean ± SE of five rats.

ministration of calcitonin, with identical significant reductions of serum calcium levels. Maximal hypocalcemic responses of 29 and 28.3% reduction, respectively, were seen between 2 and 4 hr after administration of calcitonin in both iu and sc administrations. The hypocalcemic response to iu and sc calcitonin administrations provided an indirect measure of the extent of calcitonin absorption and a direct measure of bioequivalency. The difference in the mean AUC after iu and sc administrations was found to be negligible (124 ± 13 and 115 ± 13 mg% × hr, respectively).

AUCs calculations in cervix-ligated and nonligated animals served also as a measure to assure no leak of the solution from the uterus lumen and absorption from the uterus only. The mean AUC after iu administration of calcitonin in the nonligated animals was found not to be significantly different from that found in ligated animals (105 ± 12 and 128 ± 14 mg% × hr, respectively). It was decided, therefore, to combine the data of the two groups (as presented in Fig. 4). Similar results were obtained in ovariectomized rats (101 ± 11 and 110 ± 12 mg% × hr, respectively; data not shown).

Controlled-Release Administration

Calcitonin and calcium serum levels following iu administration of calcitonin by mini osmotic pumps and polyurethane matrices are described in Fig. 5. Four days after implantation of both calcitonin matrices and mini osmotic pumps, calcitonin levels reached a plateau. However, the serum calcitonin concentration in the mini osmotic pump-treated rats was ~10 times higher than in the polyurethane matrix-treated rats. In both treatment groups serum calcium concentrations were not significantly changed.

DISCUSSION

The major findings of this study are that high MW peptides such as insulin and calcitonin are absorbed in a biologically active form from the uterus of rats. This new route of drug administration may be suitable for postmenopausal women during their menopausal period or for treating postmenopausal-related disorders such as osteoporosis.

Diffusion studies showed that the uterine tissue is readily permeable to these peptides. However, the uterine tissue was found impermeable to ionizable molecules such as methylene blue (base) and HEBP (acid), which might be due to adsorption of these agents to the tissue surface. Uterus histology did not provide an explanation for this pattern of permeability, and further studies are required in order to

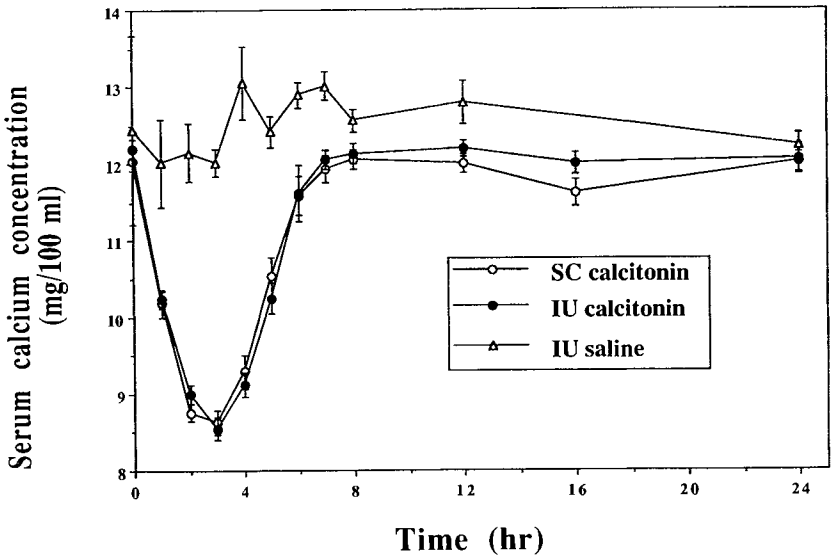

Fig. 4. Serum calcium concentration in intact rats treated with calcitonin (0.8 U/kg body weight). Each point for iu and sc calcitonin represents the mean ± SE of 11 rats (12 for iu saline). The difference in the AUC between the sc and the iu routes is negligible.

characterize the mechanism of intrauterine absorption. The permeabilities of intact and atrophied uteri were similar.

Similar profiles of serum insulin concentration were obtained by sc injection and iu delivery. Insulin absorption by both routes resulted in the same, significant reduction of serum glucose levels. These results demonstrate that iu administration of insulin is as effective as the sc route, which is commonly used in diabetic patients. Continued insulin administration for 14 days was also found to elicit hypoglycemic effect. Similarly, the iu route was found bioequivalent

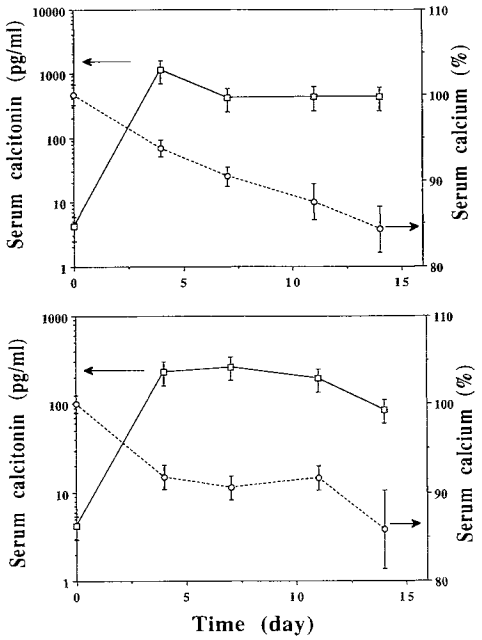

Fig. 5. Serum human calcitonin and calcium levels following mini osmotic pumps (top) and polyurethane matrices (bottom) implanted in the uterus of ovariectomized rats for 14 days. Each point represents the mean ± SE of seven rats.

(measured by biological response) to the sc route following calcitonin administration. The extent and duration of the hypocalcemic response following iu administration of calcitonin in intact and ovariectomized rats were similar to those obtained by sc injection of the hormone (13). These results clearly demonstrated that both insulin and calcitonin were absorbed in biologically active form from rat uterus.

The absorption of active insulin and of calcitonin indicates the potential of the iu route for other peptides. Peptide/protein drugs are an important new class of therapeutic agents. An iu extended-duration product could allow a reduction in dosing frequency, a corresponding improvement in patient compliance and improved therapeutic result. The iu route seems suitable for prolonged, controlled-release drug administration in selected patients.

Similar to the intravaginal route, uterine blood is drained into the inferior vena cava, hence, bypassing a possible deleterious "first-pass" effect. However, the bioavailability of the vaginal route is highly variable; potentially damaging absorption promoters are often required (3,14). The epithelium of the vagina consists of a keratinized stratified squamous epithelium, providing a tight barrier to environmental substances. In contrast, the uterine is a mucous membrane made up of simple columnar epithelium. The endometrial stroma is exceptionally rich in both arterial and venous vasculature and, also, contains many lacunes, lymphatic vessels, and glands. The stromal cell is normally a free cell not making structural contact with neighboring cells. The different structure of the uterine tissue and vascularity compared with that of the vaginal mucosa might account for the better absorption of drugs from the uterus. For example, the bioavailability of calcitonin following vaginal administration of a solution in rats has been reported recently to be only 2.8% (15). In another report (14) it was found that in the absence of any enhancer, no decrease in blood glucose levels was observed after vaginal administration of insulin in rats.

Our results suggest that the menstrual cycle in the rat does not affect uterine absorption. The rats were used randomly throughout the study and for longer periods than 4 days (in calcitonin and insulin treatments).

Calcitonin is indicated in several bone-related disorders such as Paget's disease, malignant hypercalcemia, and postmenopausal osteoporosis (16). The chronic nature of these diseases makes parenteral therapy (calcitonin is inactivated in the GI tract) considerably discomforting to the patient, especially as a number of adverse reactions accompany calcitonin injections (16,17). Recently, a nasal spray of calcitonin has been introduced (Sandoz, SW). However, the hypocalcemic response in healthy humans after nasal administration is reported to be lower than that attained by the subcutaneous route (16,18).

In summary, the extent and duration of the hypoglycemic and the hypocalcemic effects induced by iu delivery of insulin and calcitonin, respectively, were equivalent to those obtained after sc injections. The uterus of both intact and ovariectomized (atrophied uterus) rats was similarly permeable to these peptides. It is concluded that the iu administration of both insulin and calcitonin is bioequivalent to subcutaneous injection. The therapy of a number of clinically important diseases could benefit from this discovery.

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REFERENCES

1. A. K. Banga and Y. W. Chien. Systemic delivery of therapeutic peptides and proteins. *Int. J. Pharm.* 48:15–50 (1988).
2. V. H. L. Lee (eds.). *Peptide and Protein Drug Delivery,* Marcel Dekker, New York, 1991.
3. H. Okada. Vaginal route of peptide and protein delivery. In V. H. L. Lee (eds.), *Peptide and Protein Drug Delivery,* Marcel Dekker, New York, 1991, pp. 633–666.
4. A. Hoffman, G. Golomb, and A. Avramoff. Administration of high molecular weight drug to female mammals for systemic effect. Israeli patent pending No. 96944 (1991).
5. G. Golomb, M. Dixon, M. S. Smith, F. J. Schoen, and R. J. Levy. Controlled-release drug delivery of diphosphonates to inhibit bioprosthetic heart valve calcification: Release rate modulation with silicone matrices via drug solubility and membrane coating. *J. Pharm. Sci.* 76:271–276 (1987).
6. M. Kidron, H. Bar-On, E. M. Berry, and E. Ziv. The absorption of insulin from various regions of the rat intestine. *Life Sci.* 31:2837–2841 (1982).
7. T. J. Wronski. The ovariectomized rat as an animal model for postmenopausal bone loss. *Cells Mater.* S1:69–74 (1991).
8. G. Golomb and V. Ezra. Prevention of bioprosthetic heart valve tissue calcification by charge modification: Effects of protamine binding by formaldehyde. *J. Biomed. Mater. Res.* 25:85–98 (1991).
9. G. Golomb and A. Shpigelman. Prevention of bacterial colonization on polyurethane in vitro by incorporated antibacterial agents. *J. Biomed. Mater. Res.* 25:937–952 (1991).
10. G. Golomb and D. Wagner. Characterization and anticalcification effects of implantable polyurethane matrices containing amorphous dispersion of bisphosphonic acid. *Clin. Mater.* 8:33–42 (1992).
11. G. Golomb, A. Schlossman, H. Saadeh, M. Levi, J. M. Van Gelder, and E. Breuer. Bisacylphosphonates inhibit hydroxyapatite formation and dissolution *in vitro* and dystrophic calcification *in vivo*. *Pharm. Res.* 9:143–148 (1992).
12. M. L. Rocci and W. L. Jusko. LAGRAN program for area and moments in pharmacokinetic analysis. *Comp. Prog. Biomed.* 16:203–216 (1983).
13. J. M. Zanelli, R. E. Gaines-Das, and P. H. Corran. International standards for salmon calcitonin, eel calcitonin, and the Asu1-7 analogue of eel calcitonin: Calibration by international collaborative study. *Bone Mineral* 11:1–17 (1990).
14. J. L. Richardson, L. Illum, and N. W. Thomas. Vaginal absorption of insulin in the rat: Effect of penetration enhancers on insulin uptake and mucosal histology. *Pharm. Res.* 9:878–883 (1992).
15. Y. Nakada, T. Sakai, M. Miyake, Y. Adachi, and N. Awata. Vaginal absorption of sodium prasterone and human calcitonin. *J. Pharmacobio-Dyn.* 15S:29–33 (1992).
16. C. H. Chestnut III. Calcitonin. In J. A. Kanis (eds.), *Calcium Metabolism. Programs in Basic Clinical Pharmacology,* Karger, Basel, 1990, pp. 73–88.
17. L. J. Deftos and B. P. First. Calcitonin as a drug. *Ann. Intern. Med.* 95:192 (1981).
18. T. Buclin, J. P. Randin, A. F. Jacquet, M. Azria, M. Attinger, F. Gomez, and P. Burckhardt. The effect of rectal and nasal administration of salmon calcitonin in normal subjects. *Calcif. Tissue Int.* 41:252–258 (1987).