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Development and in vitro/in vivo evaluation of a colonic release capsule of vasopressin

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Summary

A colonic delivery system was developed to deliver vasopressin, a polypeptide hormone, utilizing a combination of pH-dependent and time-controlled release mechanisms. The polymers Eudragit® NE 30 D, Eudragit™ S100, and cellulose acetate phthalate were found to be suitable for this purpose. The system was designed to establish a lag time (no drug release) for a period of 6 h, thereafter, vasopressin was released at a maximum rate between 6 and 7 h which corresponds to the colonic arrival time of 6.5 h in the rat. In vivo evaluation of the delivery system in vasopressin-deficient Brattleboro rats resulted in significant reduction in urine output (C_{max} of 68.1 ± 3.7% and T_{max} of 3.7 h). Even though the pharmacological availability of the colonic delivery system vs intravenous administration was low (4.64 ± 0.59%), the mean residence time was 13-times greater for the colonic delivery system.

Introduction

In recent years, peptides and proteins have gained importance as drug entities. They are highly potent therapeutic agents, elimination half-lives are in the order of seconds to minutes, and they are rapidly degraded by enzyme systems that are present throughout the gastrointestinal tract. Therefore, the physicochemical and pharmacokinetic properties of peptides make parenteral administration (intramuscular/subcutaneous/intravenous) necessary. These methods of delivery may be acceptable in acute situations where only a limited number of injections are needed. However, they are undesirable for chronic administration since frequent injections are inconvenient and uncomfortable.

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Peroral delivery of peptides

The peroral (p.o.) route is the most convenient and preferred route of drug administration. However, p.o. delivery of peptides and proteins has not been successful. This is mainly due to the

enzyme systems that exist throughout the gastrointestinal tract. The different segments of the gastrointestinal tract, from the stomach to the colon, exhibit great differences in cell structure, membrane thickness, and enzyme activity. In general, the small intestine has the highest amount of peptidase activity (Zhou et al., 1991), whereas the colon has the lowest amounts. Hence, delivery of peptides to this region of the gastrointestinal tract where enzyme activity is low offers an attractive means for increasing absorption of peptides when given by the p.o. route.

Targeted drug delivery

In this investigation, a combination of pH-dependent and time-dependent release mechanisms were used to target the p.o. dosage form to the colon. By utilizing different types of polymer coatings, a reliable and reproducible delivery system could be developed. An enteric coating would ensure resistance to gastric fluids regardless of the variability in gastric emptying time, since enteric materials are insoluble in gastric fluids. Small intestinal transit time is constant relative to gastric emptying time and is independent of the nature of the dosage form or the nutritional state of the subject (Davis, 1985; Davis et al., 1986). Therefore, additional polymer coats can be given to establish a lag time, equal to the small intestinal transit time, during which no drug is released, until the dosage form reaches the colon. The gastric emptying time in the fasted rat is about 3.5 h, while the small intestinal transit time is about 3 h (Varga, 1976). Therefore, the objective of this study is to develop a p.o. dosage form of vasopressin with a lag time (no drug release) of 6.5 h, which corresponds to the colonic arrival time in rats.

Vasopressin

Arginine-vasopressin was selected as a model peptide drug for this investigation. It is a nonapeptide hormone with a molecular weight of 1084 (base). Deficiency of this hormone results in the condition diabetes insipidus which is characterized by excretion of large volumes of dilute urine.

Materials and Methods

Materials

Synthetic arginine-vasopressin (acetate salt) was obtained from Sigma Chemical Co. Radiolabeled arginine-vasopressin ([*phenylalanyl*-3,4,5-^{3}H(N)]vasopressin) with a specific activity of approx. 70 Ci/mmol was purchased from New England Nuclear. The radiochemical purity of [^{3}H]vasopressin was determined by thin-layer chromatography on silica gel G plates (Eastman Kodak Co.), using a mixture of *n*-butanol/pyridine/acetic acid/water (30:20:6:24) (NEN® Dupont, 1989) as the mobile phase. Minicaps® were obtained from Elanco. Minicaps® are clear, hard gelatin capsules, 2 × 8 mm, specially designed for administration to rats. ScintiVerse™ BD (Fisher Scientific Co.) was used as the liquid scintillation cocktail. The Eudragit® polymers and CAP were gift samples from Röhm Pharma and Eastman Kodak Co., respectively. Sucrose and ethanol (95% v/v, HPLC grade) were purchased from Aldrich Chemical Co. All other materials were of reagent grade and were used as received.

Preparation of capsule formulation

Minicaps® were exposed to formaldehyde vapor (37% w/w aqueous solution) for 1 h and dried at 50 °C for 2 h in an oven. To make vasopressin blends, powdered sucrose was dispersed in a solution of vasopressin (spiked with [^{3}H]vasopressin) in ethanol (95% v/v). After thorough mixing, the ethanol was evaporated under a slow stream of nitrogen gas. Sucrose was used as the diluent because this sugar has been used for many years as a stabilizing agent to maintain the biological activity of proteins and peptides (Lee et al., 1981; Arakawa et al., 1982, 1991). Table 1 gives the composition of the blend that was filled in the Minicaps®. Approx. 6 mg of the blend was filled into each Minicap®; the Minicaps® were sealed with an aqueous solution of gelatin. Screening of several Eudragit® and cellulose polymers identified Eudragit® NE 30 D, Eudragit™ S100, and cellulose acetate phthalate as suitable for coating. The filled capsules were dip-coated with an aqueous dispersion of

TABLE 1

Composition of colonic release capsules (CRC)

Active ingredient	Quantity per capsule
Vasopressin	6.0 μg
[^{3}H]Vasopressin	1.0 μCi
Sucrose	6.0 mg
Surface area of capsules	67.0 mm^2

Eudragit® NE 30 D and Eudragit™ S100 (7:3 ratio) and dried. This was followed by coating with a 10% w/w solution of cellulose acetate phthalate. The cellulose acetate phthalate solution contained acetone and methanol (45% w/w each) as solvents and 1% w/w *n*-butyl phthalate as a plasticizer.

Determination of residual formaldehyde content of capsules

The residual free-formaldehyde content of capsules exposed to formaldehyde vapor was determined spectrocolorimetrically at 490 nm (Macfadyan, 1945; Jain et al., 1984). To each formaldehyde-treated capsule, 10 ml of distilled water was added and agitated in a shaker-bath at room temperature for 1 h. 1 ml of this solution (filtered through 0.22 μm filter) was added to 9.0 ml of a 0.2% w/v solution of chromotropic acid in sulfuric acid (80% v/v). The samples were heated for 30 min on a boiling water-bath. The intensity of color produced was measured spectrophotometrically after the samples had cooled to room temperature. The limit of detection of residual free formaldehyde was 0.1 μg/ml.

In vitro dissolution of capsules

The in vitro release from capsules was determined by dissolution tests using USP Method I at 37 ± 0.1°C at 50 rpm. Simulated gastric fluid (900 ml), without enzymes, was used as the dissolution medium. To simulate the in vivo pH-time profile, samples were withdrawn at 1-h intervals and replaced with an aqueous solution of anhydrous dibasic sodium phosphate (7.67% w/v) and sodium hydroxide (2.16% w/v). Fig. 1 demonstrates a profile of the in vitro pH-time changes.

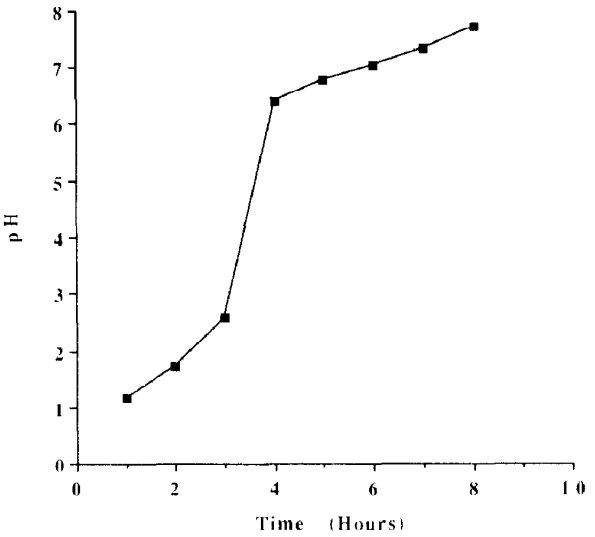

Fig. 1. In vitro pH-time profile.

Vasopressin samples were analyzed by liquid scintillation counting after mixing 1 ml of the sample with 5 ml of liquid scintillation cocktail.

In vivo evaluation

The colonic release capsules (CRCs) were evaluated in vivo in vasopressin-deficient Brattleboro rats (Harlan). Seven rats were used in a crossover design with a washout period of 1 week between studies. The rats were housed individually in metabolism cages at 22–25°C. Due to vasopressin deficiency, these rats excrete large volumes of urine, equal to 70% of their body weight, in contrast to 3% in normal rats. Exogenous administration of vasopressin corrects the excessive excretion of urine. The correlation between pharmacologic response and plasma drug concentration is not well established for most peptide drugs (Gloff et al., 1990). Hence, pharmacologic response of peptides is a more accurate reflection of the performance of peptide delivery systems than plasma concentrations. Therefore, the efficacy of the CRCs was determined by measuring the reduction in urine output following administration of the dosage form. Urine output was determined for 1 week before starting a study to establish baseline values (expressed as ml/100 g body weight).

The rats were fasted for at least 18 h prior to the study, but water was available ad libitum. Each rat was given one CRC via a plastic tube and stylet (lubricated with glycerin) under ether anesthesia. This was followed by 1 ml of distilled water. The rats were put back in the cages, and urine samples were collected periodically until the urine output was back to baseline values. With each urine sample, volume and total radioactivity excreted in the urine were measured. The urine output was expressed as ml/100 g body weight and compared with baseline values to compute the percent reduction in urine output. Radioactivity was measured by liquid scintillation counting of 500 μl or less of urine, depending on the volume of sample. Since vasopressin is known to be released under stress conditions, placebo CRCs were given to the rats in a control study and urine samples were collected periodically.

In order to determine the pharmacological availability of the CRCs, an intravenous (i.v.) study of vasopressin was performed. 200 μl of vasopressin solution in normal saline (160 ng/ml, spiked with 10 μCi/ml) was administered i.v. into the tail vein of the rat under ether anesthesia. Urine samples were collected periodically until the output was back to baseline values and blood samples (75 μl) were taken (by clipping the tail) at 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1, 1.25, 1.5, 1.75, and 2 h after administration. As a control, 200 μl of normal saline was given i.v. Blood samples were analyzed for total radioactivity by digestion with Scintigest™ tissue solubilizer (Fisher Scientific Co.) and decolorization with a benzoyl peroxide solution (20% w/v in toluene). A known aliquot was counted after adding liquid scintillation cocktail.

Results and Discussion

All data are expressed as means ± S.E. In the preliminary studies, coating of gelatin capsules, particularly with aqueous dispersions, was seen to result in deformation of the capsule. Therefore, to facilitate application of polymer coats, empty gelatin capsules were first exposed to formaldehyde vapor. Formaldehyde cross-linked the gelatin, thereby enhancing the coating procedure. Fig. 2 shows the release profiles of plain gelatin capsules of vasopressin and capsules exposed to formaldehyde. Due to cross-linking dissolution of the gelatin is hindered; therefore, formaldehyde-treated capsules released vasopressin gradually over a period of 1 h, while the untreated capsules dissolved in 10 min. The residual free-formaldehyde content was below the detection limits for all capsules tested.

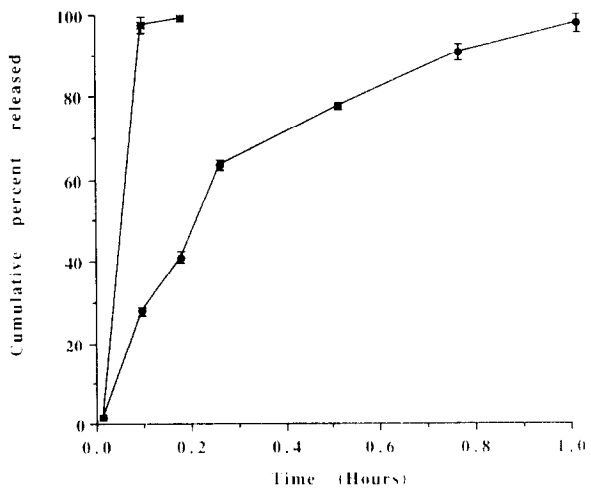

Fig. 2. Dissolution profile of vasopressin capsules (means ± S.E., $n = 6$). gelatin capsules (×) and capsules exposed to formaldehyde (●).

Fig. 3 demonstrates the dissolution profile of formaldehyde-treated capsules, with cellulose acetate phthalate coats (6.53 ± 0.47 mg/cm^2) and without coats. It is seen from this graph that cellulose acetate phthalate prevents the release of the drug for 4 h, i.e., until the pH is about 6.4. This corresponds to a gastric emptying time of about 3.5 h in the rat. Between 4 and 5 h, i.e., between pH 6.4 and 6.8, 100% of the drug is released. To further delay the release of drug from the capsules, additional coats were given with a mixture of Eudragit® NE 30D and Eudragit™ S100 (7:3). Eudragit™ S100 is an anionic polymer of methacrylic acid and methylmethacrylate; it dissolves in intestinal fluids from pH 7.0. Eudragit® NE 30 D is a neutral polymer of ethacrylate and methylmethacrylate. It is not

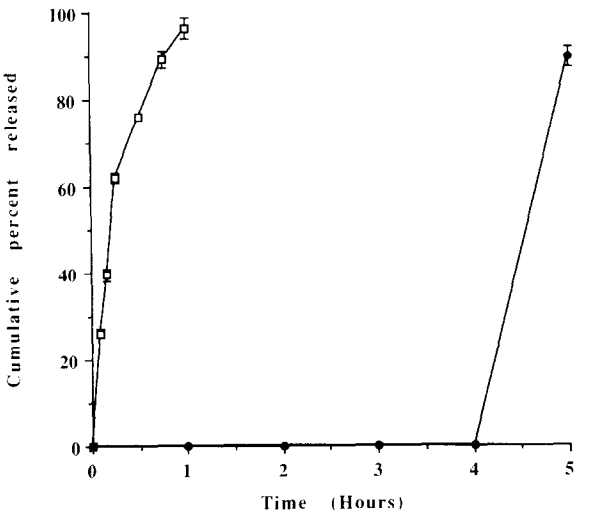

Fig. 3. Dissolution profile of formaldehyde-treated vasopressin Minicaps®, with (●) and without (□) CAP coating (means ± S.E., $n = 6$).

soluble in water; however, it is permeable and swellable in water. It has been reported that a 7:3 ratio of Eudragit® NE 30D and Eudragit™ S100 is a satisfactory combination for sustained release from capsules (Lehmann, 1989). Fig. 4 gives the dissolution profiles of formaldehyde-

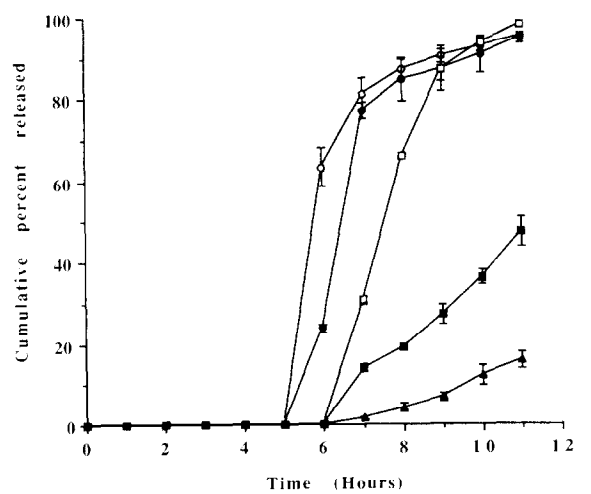

Fig. 4. Release profiles of formaldehyde-treated vasopressin Minicaps®, coated with CAP (6.55 ± 0.32 mg/cm^2) and increasing amounts of Eudragits® (Means ± S.E., $n = 4$): 2.05 ± 0.042 (○); 2.55 ± 0.046 (●); 3.06 ± 0.078 (□); 3.56 ± 0.039 (■); 4.07 ± 0.056 (▲) mg/cm^2.

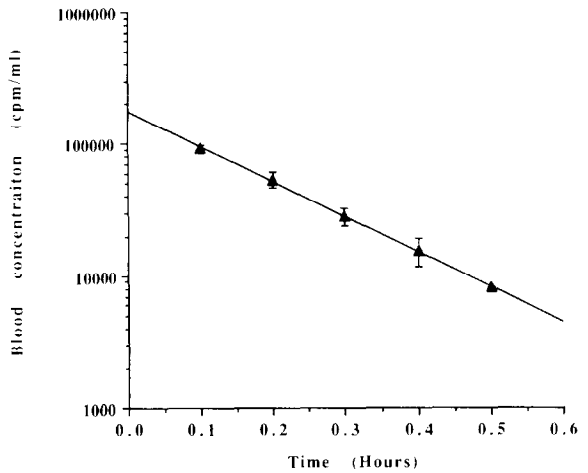

Fig. 5. Blood concentration-time profile (means ± S.E., $n = 7$) following i.v. administration of vasopressin in Brattleboro rats (dose = 0.032 μg).

treated capsules, coated with increasing amounts of Eudragit® and a fixed amount of cellulose acetate phthalate (6.55 ± 0.32 mg/cm^2). It was observed that the amount of drug released from the capsules decreased on increasing the thickness of the Eudragit® polymer coats. The formulation with 3.06 ± 0.115 mg/cm^2 of Eudragits® and 6.55 ± 0.32 mg/cm^2 of cellulose acetate phthalate resulted in a lag time of 6 h. Following this, vasopressin was released at a maximum rate between 6 and 7 h which corresponds to the colonic arrival time in the rat. Hence, this formulation was considered suitable for in vivo evaluation.

TABLE 2

Kinetic parameters of vasopressin following intravenous administration to Brattleboro rats (n = 7)

Parameters	Mean (S.E.)	
Dose (ng)	32.0	(0)
Body weight (kg)	0.169	(0.011)
λ_z (h^{-1})	6.047	(0.237)
$t_{1/2}$ (h)	0.119	(0.005)
AUC(0–∞) (cpm ml^{-1} h)	29406	(883.7)
AUMC(0–∞) (cpm ml^{-1} h^2)	4562.7	(208.8)
MRT (h)	0.156	(0.007)
Cl_{tot}/f (ml min^{-1} kg^{-1})	6.628	(0.596)
V_{dbeta}/f (l kg^{-1})	0.067	(0.008)

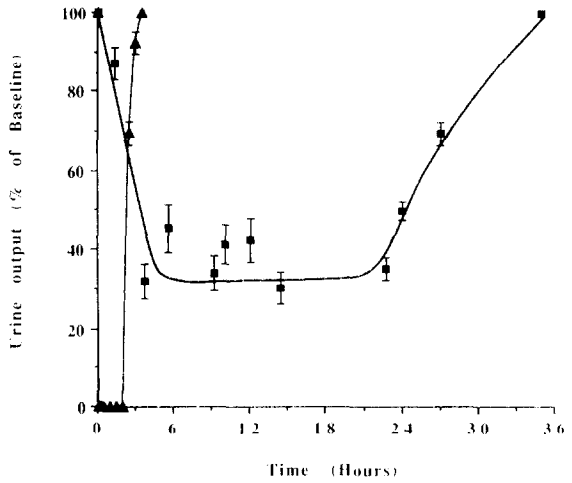

Fig. 6. Comparison of urine output (% of baseline) (means + S.E., $n = 7$) following i.v. (▲) and CRC (■) administration in Brattleboro rats (dose = 0.032 ± 0 μg and 5.67 ± 0.27 μg, respectively).

Fig. 5 depicts the blood concentration-time profile obtained following i.v. administration of vasopressin to Brattleboro rats. The pharmacokinetic parameters of vasopressin (Table 2) were determined via two computer programs: RESID (Ritschel, 1975) and AUC-RPP (Ritschel, 1986). The elimination half-life was very short, 0.116 ± 0.012 h (6.95 ± 0.724 min). This corresponds with the reported literature values of 10–20 min in humans (Drug Information, 1989). The urine output (% of baseline) following i.v. and CRC administration is shown in Fig. 6. The i.v. route resulted in a dramatic reduction in urine output (100% reduction); however, this effect lasted only for about 2 h. The urine output was back to baseline values in 3.5 h. The CRC resulted in a C_{max} of 68.1 ± 3.7% (with a T_{max} of 3.7 h). Subsequently, the urine output fluctuated between 30 and 50% of baseline until 24 h. Administration of placebo CRC did not change the urine output from baseline values.

No response was expected until the CRC reached the colon, i.e., for about 6.5 h. The shorter T_{max} was probably the result of a rapid gastric emptying time, since the rats were fasted for at least 18 h prior to the p.o. study. It is also likely that vasopressin was being absorbed from the small intestine, which would explain the decrease in urine output observed in the first 3 h. Large intestinal transit time in the rat is reported to be about 23 h (Varga, 1976). It is likely that the CRC resided in the large intestine for about 20 h; slow diffusion through the viscous contents of the large intestine may have resulted in prolonged urine reduction effects. This is a possible explanation for observing a 30–50% reduction in urine for a period of 24 h.

Fig. 7 shows a plot of mean urinary excretion rate (cpm/h) vs midpoint time (h) on a semi-logarithmic scale. The excretion rate increased steadily and remained fairly constant between 6 and 20 h; the reduction in urine output was also seen to be steady during this time interval. The area under the urine reduction-time curve (AUC) and area under the first moment curve (AUMC) were calculated by the trapezoidal rule. The mean residence time (MRT) was computed as the ratio of the AUMC to AUC. Pharmacological availability was calculated as:

$$P.A. = \frac{AUC_{CRC} \cdot (dose/BW)_{I.V.}}{AUC_{I.V.} \cdot (dose/BW)_{CRC}}$$

The results are presented in Table 3. Even though the pharmacological availability was low,

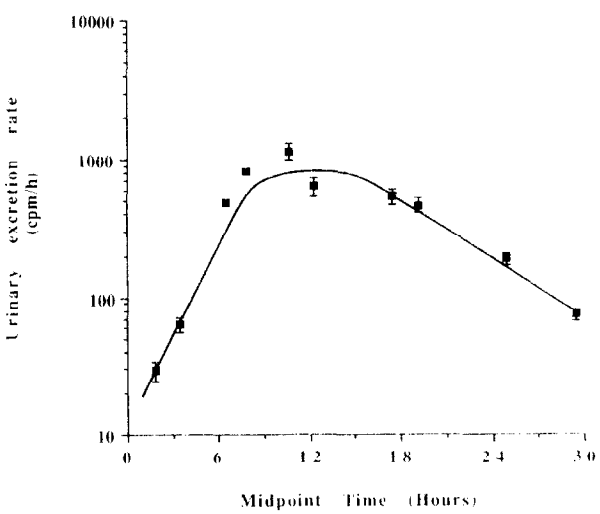

Fig. 7. Urinary excretion rate vs midpoint time following administration of CRC to Brattleboro rats (means + S.E., $n = 7$) (dose = 5.67 + 0.27 μg).

TABLE 3

Parameters obtained from the area under the urine reduction-time curve (means ± S.E., n = 7) (UR, urine reduction)

Parameters	i.v.	CRC
Body weight (kg)	0.164 ± 0.01	0.186 ± 0.006
Dose (μg)	0.032 ± 0	5.67 ± 0.10
AUC (% UR h)	239.4 ± 4.20	1779.2 ± 243.3
T_{max} (h)	0	3.7
C_{max} (% UR)	100 ± 0	68.1 ± 3.7
AUMC (% UR h^2)	611.9 ± 23.5	61692 ± 11583
MRT (h)	2.55 ± 0.05	33.3 ± 2.63
Pharmacological availability (%) (CRC vs i.v.)	100	4.64 ± 0.59

the MRT was 13-fold greater with the CRC as compared with i.v. administration. To achieve the same MRT, a dose of 0.42 μg of vasopressin would be required i.v., i.e., a dose greater than 10 times the normal i.v. dose.

In this study it is very likely that some of the vasopressin was inactivated in vivo at the site of release from the CRC. Even though the colon has low peptidase levels, it still has some finite quantities of peptidases. This may have been sufficient for the degradation of vasopressin which was present in very small quantities (6 μg). Incomplete release from the CRC could also have resulted in low pharmacological availability. Another contributing factor could be the presence of bacteria and fecal matter in the colon; the effects of these factors on the stability of peptides is not well documented. Vilhardt et al. (1983) demonstrated that in water-loaded conscious Beagle dogs intragastric administration of 5 μg of vasopressin did not give a detectable antidiuretic response, while 50 μg of vasopressin produced a marked (100% urine reduction) but very short (15–30 min) antidiuretic response. The study of Saffran et al. (1988) reported a weak antidiuretic response (30% urine reduction for about 40 min) in Sprague Dawley rats following administration of p.o. solutions of vasopressin. Therefore, in comparison the pharmacological availability achieved in this study with the CRC in Brattleboro rats is a significant improvement over that attained with p.o. solutions of vasopressin.

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