in solid formulations only after sustained heating at 60°C after a prolonged storage time (5).

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Controlled Long-Term Release of Small Peptide Hormones Using a New Microporous Polypropylene Polymer: Its Application for Vasopressin in the Brattleboro Rat and Potential Perinatal Use

JENNEKE KRUISBRINK and GERARD J. BOER ˣ

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Abstract □ Based on drug release by microporous hollow fibers and the recent introduction of microporous polymers, a new technique was developed for controlled delivery of peptides. Small-diameter microporous polypropylene tubing, lumen-loaded with microgram quantities of vasopressin, and coated with collodion, releases vasopressin after *in vitro* immersion slowly (1–100 ng/d) and constantly for months. The mechanism of pseudo-zero-order delivery is based on high adsorption of vasopressin, keeping the void volume concentration of dissolved vasopressin constant, which is consequently a constant driving force of outward diffusion. The collodion coating prevents the entry of proteinaceous compounds which would result in rapid desorption of vasopressin. The present delivery module provides a lasting release for other peptides as well (lysine-vasopressin, oxytocin, α-melanocyte-stimulating hormone and, to a lesser extent, Met-enkephalin). The microporous polymer-collodion device is biocompatible and, loaded with vasopressin, successfully alleviates the diabetes insipidus of Brattleboro rats deficient for vasopressin. Subcutaneous implantation normalized diuresis for a period of 60 d and constant urine vasopressin excretion is observed. When the commercially available osmotic minipump is too large for implantation, the small size of the present controlled-delivery system allows peptide treatment of young and immature laboratory rats, even if located *in utero*.

Keyphrases □ Controlled drug delivery—vasopressin, microporous polymers, Brattleboro rat □ Vasopressin—controlled drug delivery, microporous polymers, Brattleboro rat □ Brattleboro rat—vasopressin, controlled delivery, microporous polymers

Peptide hormones and many other substances must be administered in a long-term continuous fashion and within a specified range of concentrations in order to bring about physiological changes. Effective dosages can be given by injections, but because of the short half-life of hormone peptides, high doses often have to be administered, resulting in a sawtooth pattern of peptide levels. As a consequence, side effects frequently occur. Several controlled-release techniques have been developed, ensuring a continuous and constant application for a variety of substances (1–5). Most of these techniques are based either on the principle of: (*a*) constant diffusion of substances (mostly hydrophobic steroids and drugs) through polymer matrices (6) or (*b*) constant delivery of a liquid volume containing the physiologically active agents from an osmotically active core (3). A widely used osmotic minipump—an example of the second approach—has the advantage that it is suitable for all types of compounds (including peptide hormones). However, because of its size, implantation cannot be performed in small laboratory animals or fetuses.

Recently we encountered problems when vasopressin supplementation had to be given to vasopressin-deficient newborn Brattleboro rats (7). Based on the possibilities of drug release by the use of hollow fibers (6) and the recent introduction of a new microporous polymer matrix[1] (8, 9), a technique has now been developed through which controlled delivery of peptides can be obtained. It is small enough to allow the use in immature rats. Preliminary results both on the development and on the applications of the present technique have been partially in-

[1] Accurel polypropylene.

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corporated in other publications (10–12); this paper describes the method in full detail.

EXPERIMENTAL SECTION

Materials—The new microporous polymer matrix[1] has a large void volume, consisting of cells interconnected by small pores (8, 9, 12). In the present study, polymer matrix polypropylene tubing[2] (P 78/15/2; wall void volume 71%, cell and pore size of ~5 and 0.5 μm, and an o.d. and i.d. of 1.6 and 0.9 mm, respectively) was used. Vasopressin[3] (465 U/mg), lysine-vasopressin, Met-enkephalin[5], oxytocin[6], α-melanocyte-stimulating hormone[6], and [^{3}H]-Met-enkephalin[7] (50 Ci/mmol) were obtained commercially. [^{125}I-Tyr]-vasopressin was prepared (13) with a specific activity of 0.5–1.0 Ci/mmol.

Test Tube Experiments—Microporous polymer tubing (2 cm; one end heat-sealed with a mini-soldering iron) filled with ethanol (a few minutes under reduced pressure) was either immersed in an aqueous vasopressin solution for wall-loading (1 mg/mL, 24 h) or in water (1 h) for lumen-loading using a syringe (7 or 22 μg of vasopressin in 7.5 μL). The tubing was then mounted on a serum filter tube so that 1.5 cm of the closed end was immersed or, after heat-sealing the other end, was completely immersed. In the final stage a lumen-loaded double-closed preparation was enfilmed with collodion[8]: the tubing was dipped three times in the collodion solution [0.05 g/mL ether–ethanol, 3:1 (v/v)] and air dried between dippings (12). As a control for this final procedure, vasopressin was made in 2% agar and sucked into silicon tubing containing a strand of suture silk running through its lumen. A 1.5-cm rod was prepared with the outer dimensions of the microporous polymer tubing which could also be coated with collodion (6 dips).

Release of vasopressin was followed at 37°C in 1 mL of distilled water 10% swine serum containing 0.01% sodium azide or 0.5% bovine serum albumin. The volume was replaced daily. When release during 1 d was followed, 25-μL samples were drawn. Samples were stored at −20°C until an RIA for vasopressin was carried out (14, 15). Degradation of the vasopressin immunoreaction in water, serum, and albumin media (water, 0.5 ng–0.5 mg/mL; albumin and serum, 7 ng/mL) was determined during 24-h incubation at 37°C.

Microporous polymer-collodion devices were prepared (containing 7 μg of lysine-vasopressin, oxytocin, and α-melanocyte-stimulating hormone) and daily release was followed radioimmunologically in 10% serum (14–16). The tritiated form of Met-enkephalin (7 μg; final specific activity, 1.2 μCi/mmol) was applied and the release was followed by counting radioactivity.

Animal Experiments—Homozygous (HOM) Brattleboro rats (~140 g), congenitally deficient for vasopressin[9] (17), were kept under standard conditions in metabolism cages (18). After a control period of 3 d, in which the rats showed diabetes insipidus, the animals received subcutaneously, under light ether anaesthesia, microporous polymer-collodion tubing loaded with 7.5, 22, or 220 μg of vasopressin. Vasopressin release was followed in duplicate animals by measuring daily urine production and osmolality and by the assay of urine-excreted vasopressin (see above).

For comparison, single HOM Brattleboro male rats received: a 22-μg vasopressin-loaded microporous polymer without collodion enfilming; a 22-μg vasopressin-agar-collodion preparation; a minipump[10] filled with 22 μg of vasopressin in saline (pumping rate was 0.4 μL/h, *i.e.*, 1000 ng/d); or daily subcutaneous injections of vasopressin tannate[11] (0.5 U/100 g). Finally, a male Wistar rat received a microporous polymer-collodion implant containing 22 μg ^{125}I]-vasopressin (1 mCi/mmol), to follow urine excretion of iodine-125 (drinking water contained 0.1% KI).

Perinatal Implantations—HOM Brattleboro pups (age, 5 d) (7) received subcutaneously a water-filled microporous polymer-collodion tubing (1.5 cm) with a 0.7-mm diameter (P 17/80/2) or the dimensions used above. The largest tubing, placed longitudinally to the animal's spine, was checked daily by palpation, and, at 1 month of age, all pups were sacrificed and an attempt was made to locate the tubing.

The uterus of pregnant (18th day of gestation) Wistar rats was exposed for fetal operation according to Swaab and Honnebier (19). A small diameter microporous polymer-collodion preparation, 0.5- or 1-cm in length inserted into a 19-gauge needle, was placed through the uterine wall underneath the skin of the back of the fetus. Implants were performed on several fetuses within the exposed uterus. Ten days after birth the offspring were sacrificed and the location of the microporous polymer implant was examined.

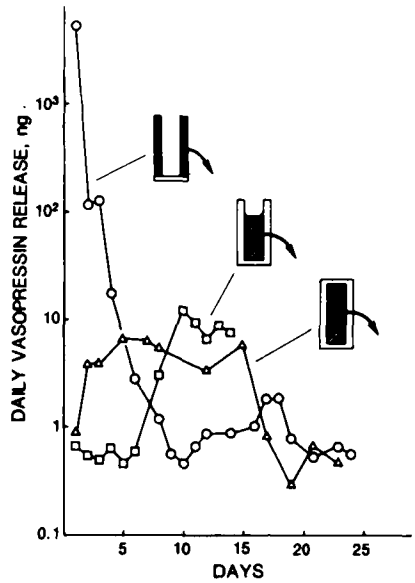

Figure 1—*Vasopressin levels measured in daily renewed 1-mL water volumes containing a microporous polymer polypropylene tubing loaded with the drug (shaded area). Data were taken from single representative experiments. Key: (○) 21 μg, wall-filled end-sealed tubing; (□) 15 μg/5 μL, lumen-filled end-sealed tubing; (△) 7 μg/7.5 μL, lumen-filled double end-sealed tubing.*

RESULTS

Test Tube Experiments with Vasopressin—From the different loading procedures of the microporous polymer polypropylene tubing, the most continuous and constant release of vasopressin on water immersion was reached after lumen loading with double-end-closed tubing (Fig. 1). On wall filling, vasopressin ran out almost asymptotically whereas the lumen-filled open

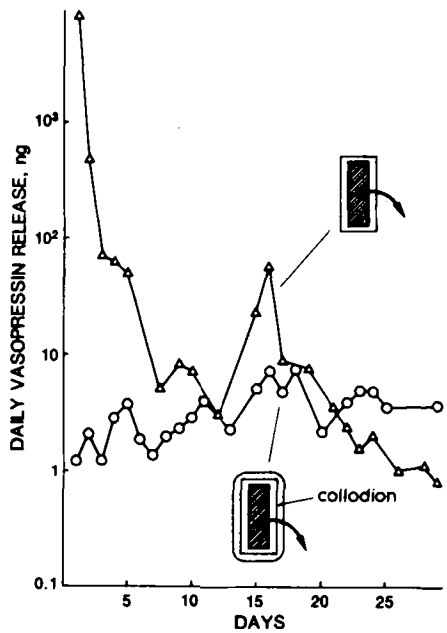

Figure 2—*Effect of collodion coating on vasopressin level in daily renewed 10% swine serum on immersion of a vasopressin-microporous polymer-collodion preparation (7 μg). Key: (△) without collodion; (○) with collodion; (▨) drug.*

[2] Kindly supplied by Mr. D. Heitmann (ENKA Research Institute, Obernburg, FRG).
[3] Grade VIII, lot 99C-1352; Sigma, St. Louis, Mo.
[4] Grade IV; Sigma.
[5] UCB, Brussels, Belgium.
[6] Organon, Oss, The Netherlands.
[7] NEN, Doorn, The Netherlands.
[8] Parlodion; Schuchardt.
[9] CPB/TNO, Rijswijk, The Netherlands.
[10] Model 2002; Alzet.
[11] Pitressin tannate; Parke-Davis.

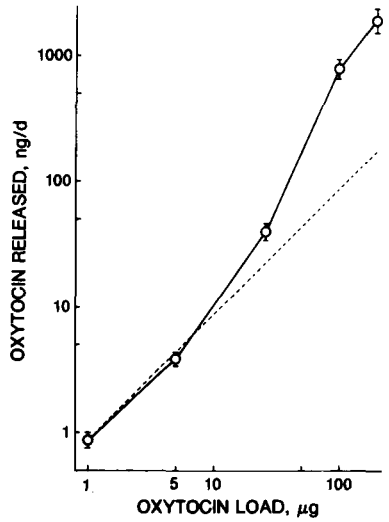

Figure 3—*Daily release of oxytocin from microporous polymer polypropylene-collodion tubing as a function of the amount of oxytocin brought into the tubing lumen. The medium is 1 mL of 0.5% albumin, renewed daily. Data are from duplicate incubations assayed over a period of 30 d. Vertical bars indicate* ± SEM; *the dashed line is the linear relationship.*

tubing had a 5-d delay before a period of rather constant release. The enveloped vasopressin preparation total release was small: ~1% of the original vasopressin load after 2 weeks. Since aqueous vasopressin loses only 20% of its activity during the same period, a strong adsorption of vasopressin onto the large internal polypropylene surface (~100 m^2/g) (9) was presumed. This contention was supported by the 300% accumulation of radioactivity of the microporous polymer polypropylene after immersion for 1 h in a ^{125}I-vasopressin solution, and the enhancement of total release (≤70%) asymptotically when surface active (0.05% Triton X-100) and/or absorption-competitive proteinaceous compounds (concentration dependent from 0.025 to 1.0% gelatin) were coloaded with vasopressin (not shown). Since incubation in 10% serum (*i.e.*, entry of externally present proteinaceous compounds) showed the rapid depletion of vasopressin as well (Fig. 2), it was therefore necessary for *in vivo* application to abolish or suppress the effect of external agents. Enveloping the vasopressin-microporous polymer preparation with cellulose dialysis membrane or an exclusion filter[12] (both permeable to vasopressin) did not prevent the asymptotic release peak (data not shown). However, after coating with nitrocellulose (collodion), the constant release of vasopressin reappeared in serum solution (Fig. 2). This enfilming was therefore applied in all subsequent studies. With a 7-μg load, daily released amounts of 2–10 ng of vasopressin were found for at least 50 d. Higher loads increased the release rate, but the relation between loading and average first month daily release shows a progressive enhancement for the heterologous oxytocin (Fig. 3). For the highest load (200 μg), constancy of release was lost; instead, a slowly asymptotically decreasing release was seen over the 30-d period.

Concentration of vasopressin in the release medium as a function of time during the first 3 d of immersion and on day 15 showed similar daily logarithmic curves (maximum reached by 6 h in serum, Fig. 4). Incubations in 0.5% albumin, started in the presence of external vasopressin (≤200 ng/mL) showed that with external concentrations <50 ng/mL, a release of vasopressin

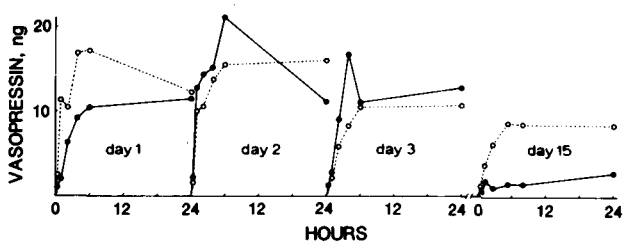

Figure 4—*Daily time course of vasopressin content in 1 mL of 10% serum on immersion of a vasopressin-microporous polymer-collodion device (7 μg) during the first 3 d and on day 15 (duplicate incubations).*

[12] Amicon UM-2.

Table I—Release and Uptake of Vasopressin by Microporous Polymer–Collodion Tubing in an Albumin Medium [a]

	Vasopressin in Medium, ng/mL	Vasopressin in Medium (by RIA), ng/mL	
		0 h	24 h
Vasopressin-microporous polymer			
	200	187 ± 16	66 ± 13
	100	93 ± 9	59 ± 9
	50	40 ± 7	31 ± 7
	10	6.1 ± 0.5	34 ± 7
	0	0	30 ± 5
Medium [b]	200	197	164
	50	42	37

[a] Tubing loaded with vasopressin (7 μg) in a 0.5% albumin medium at 37°C. Data ± *SEM* are averages of duplicate findings assayed on 3 subsequent days ($n = 6$). [b] Duplicate assays were performed on a single day for the medium, showing no marked degradation of vasopressin.

is seen; with external concentrations >50 ng/mL, vasopressin is taken up from the medium by the microporous polymer device (Table I).

The mean thickness of the collodion layer around the tubing was 45 μm (*SEM*, 2) as measured under a microscope in random 30-μm cryostat cross sections of eight preparations. A collodion bag of similar shape and thickness (42 μm, *SEM*, 2; measured in three preparations) around vasopressin in agar shows a total wash-out of vasopressin in 10% serum within a few days, while without collodion this occurs even more rapidly (data not shown).

Test Tube Experiments With other Peptides—The *in vitro* results with vasopressin were similarly obtained with lysine-vasopressin- and oxytocin-loaded microporous polymer-collodion devices, while the release pattern of α-melanocyte-stimulating hormone showed an initial lag-phase of 5 d (Fig. 5). Met-enkephalin was not adsorbed to the polypropylene (in a water medium, 70% of the load released asymptotically within 4 d) but when microporous polymer tubing was covered with collodion, the release rate was considerably retarded.

Application in the Brattleboro Rat—Immediately after implantation of the vasopressin-microporous polymer-collodion preparation, diuresis decreased from 60–70 mL of urine/100 g of body weight/d to normal (heterozygous) levels of 5–15 mL. Urine osmolality concomitantly increased from 200 to >1000 mOsm/kg H_2O (Fig. 6 A–C; Table II). A load of 7.5 μg of vasopressin reduced diuresis for 30 d, after which it slowly reached pre-implantation diabetes insipidus values at day 60. The period of low urine production increased to 35–45 and 50–80 d for the 22- and 220-μg vasopressin-filled microporous polymer tubing, respectively, with urine osmolalities of ~1500 mOsm/kg of H_2O (Table II). Return to preimplantation values became immediate after surgical removal of the implants (Fig. 6B, C). The removal was carried out under ether anesthesia. The connective tissue enveloping the implant was not attached and no signs of infection or growth abnormalities were seen.

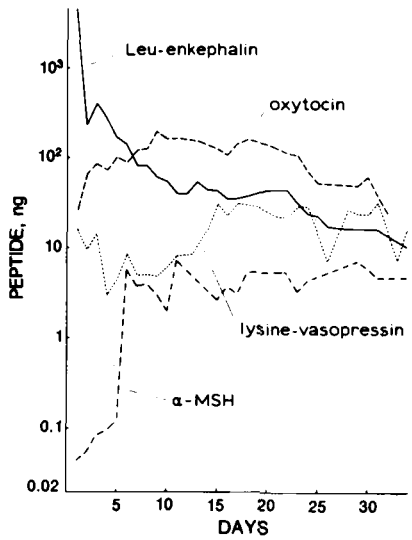

Figure 5—*Daily* in vitro *release in 10% serum medium of several neuropeptides from the microporous polymer-collodion delivery module filled with 7 μg/7.5 μL solutions.*

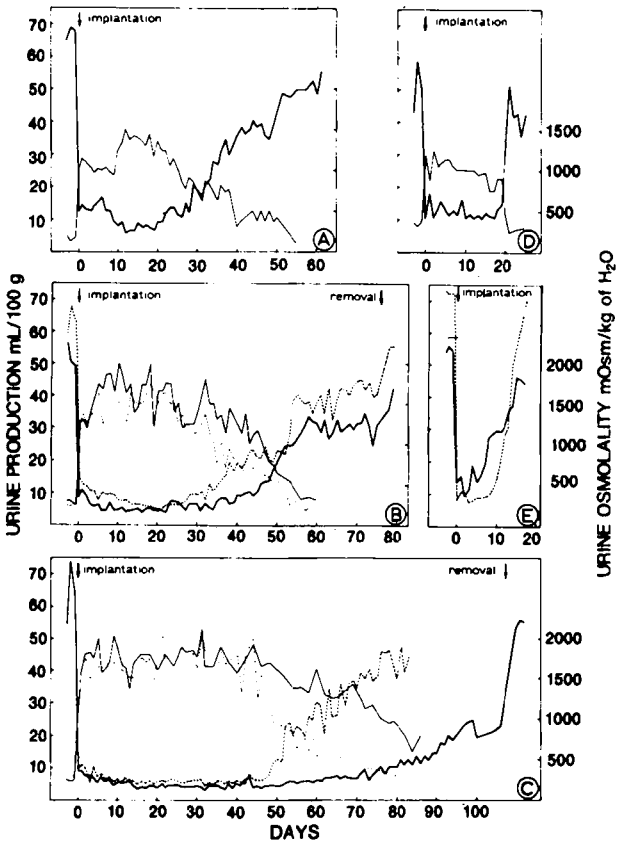

Figure 6—*Urine production (thick lines) and osmolality (thin lines) in male Brattleboro rats after implantation of different vasopressin-releasing preparations. Key: (A–C), duplicate subcutaneous implants of a microporous polymer-collodion device loaded with 7, 22, and 220 μg of vasopressin respectively (removal of the implant is indicated in B and C); (D) subcutaneous implanted osmotic minipump loaded with 22 μg of vasopressin (release rate, 1 μg/d); (E) implantation of a microporous polymer device without collodion enfilming (——) or of an agar cylinder with collodion ensheathment (·····) containing 22 μg of vasopressin.*

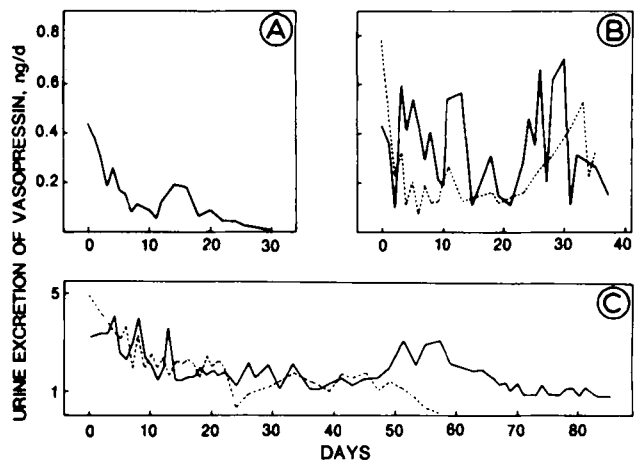

Figure 7—*Vasopressin excretion in the daily urine of Brattleboro rats after implantation of vasopressin-microporous polymer-collodion preparations containing 7 (A), 22 (B), and 220 μg of vasopressin (C) (Figs. 6A–C).*

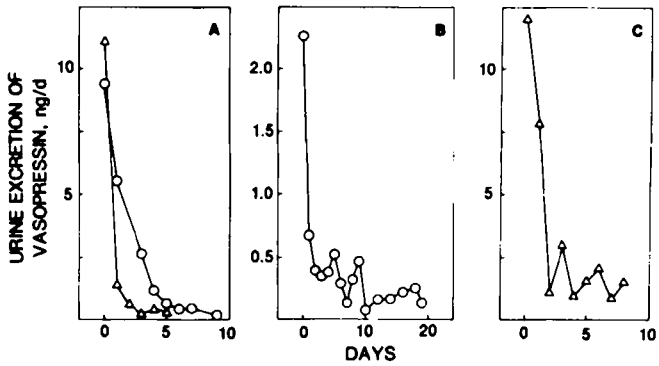

Figure 8—*Vasopressin excretion in the daily urine of Brattleboro rats after implantation of (A) a vasopressin-microporous polymer device with no collodion (△) and a vasopressin-agar-collodion preparation (○) (both with a 22-μg load; cf. Fig. 6E) and (B) an osmotic minipump (1 μg/d; cf. Fig. 6D), and after (C) daily vasopressin tannate injections (0.5 U/100 g of body weight).*

The implantation of an agar-collodion preparation and a microporous polymer tubing without collodion, both containing 22 μg of vasopressin, gave only short periods of low urine production (Fig. 6E). An osmotic minipump filled with 22 μg of vasopressin alleviated the diabetes insipidus for 20 d but not to control levels. (Fig. 6B, D; Table II).

The use of the microporous polymer technique gave reasonably constant amounts of vasopressin in daily urine during the periods of constant low diuresis (Fig. 7). Daily excreted quantities increased with the load of vasopressin, but not proportionally (Table II). Vasopressin excretion using microporous polymer without collodion or an agar-collodion preparation showed rapidly decreasing curves toward undetectable levels (Fig. 8A). Excretion of vasopressin in the first few days appeared to be somewhat higher (Fig. 7), which was also observed when the osmotic minipump or daily injections of vasopressin tannate were used (Fig. 8B, C).

To quantify the actual *in vivo* release, microporous polymer-collodion tubing filled with ^{125}I-vasopressin (22 μg) was used. Daily excretion of radioactivity with the urine was ~1% for 40 d (compare with Fig. 7B). After removal of the implant, 47% of its radioactivity was still present, giving an almost complete recovery of the label.

Perinatal Implantation Trials—Five-day-old pups always survived the implantation well, but the 1.5-cm microporous polymer preparation did not allow the sutured incision to heal if the tubing was not placed at a safe distance

Table II—Diuretic Changes and Urine Vasopressin Excretion of Homozygous Brattleboro Rats After Subcutaneous Implantation of Vasopressin Delivery Devices

	Load, μg	Period, d[a]	Diuresis, mL/100 g/d	Urine Osmolality, mOsm/kg	Vasopressin Urine Excretion, ng/d
Microporous polymer	7.5	30	10.8 ± 0.6[e]	1140 ± 40[e]	0.16 ± 0.03[e]
	22	35–46	7.3 ± 0.3[e]	1430 ± 30	0.30 ± 0.04
	220	52–82	6.2 ± 0.2	1550 ± 30	1.9 ± 0.1[e]
Minipump	22	20	12.6 ± 0.5[e]	1000 ± 30[e]	0.40 ± 0.12
Vasopressin tannate[b]	—	10	5.4 ± 0.4	1270 + 40[e]	1.6 ± 0.3[e]
Pre-operative values[c]			67.5 ± 2.6[e]	255 ± 25[e]	—[f]
Heterozygous controls[d]			5.8 ± 0.7	1440 ± 60	0.44 ± 0.09

[a] Period of the drug-polymer-implanted animals taken as the period of urine output <12 mL/100 g/d and urine osmolality >800 mOsm/kg (Fig. 6). The first day peak values were not taken for the calculation of average values (see text and Fig. 6). [b] Injections (0.5 U/100 g) for 10 d. [c] Mean of all Brattleboro rats used (n = 10), measured 3 d prior to vasopressin treatment. [d] Nineteen animals taken from a prior study (7). [e] Significantly different from heterozygous rat control data, using Student's t test ($p < 0.05$). [f] Not detectable.

from the incision. Consequently, the implant was lost. Occasionally, skin tension at the site of the rough and hard ends of the implant caused injuries, and the implant was lost. However, when still present after 6 d, it remained *in situ* without further problems. These problems were not encountered when we used small-diameter (0.7 mm) microporous polymer tubing.

The subcutaneous deposition of a small implant into rat fetuses (18th day of gestation) by needle penetration through the uterus, chorion, and amnion appeared to be possible, since delivery normally took place 4 d later. The pups were sacrificed 10 d after birth and of 10 implants, 4 were localized subcutaneously, 3 were found between the rib muscles, and 3 were lost.

DISCUSSION

Mechanism of the Microporous Polymer–Collodion Delivery Module—In this study a long-lasting and rather constant release of vasopressin from microporous polymer polypropylene-collodion preparation was realized. Apparently the vasopressin, adsorbed on the large internal surface of the microporous polymer polypropylene, forms a stock from which it is gradually freed on immersion. The constant release period might be explained by a small change in the adsorption equilibrium-determined concentration of vasopressin in the void volume of the tubing, since only small amounts of vasopressin are released. This constant concentration consequently will be a constant drive for outward diffusion causing the pseudo-zero-order release rate. Such a mechanism is reinforced by: the logarithmic time release in a constant volume (the release stops when inside and outside aqueous concentrations are the same, Fig. 4); and the increase and decrease of external vasopressin concentration to a fixed level on immersion of a (high) vasopressin-loaded device in solutions of, respectively, lower and higher vasopressin (release or uptake until equilibrium is reached, Table I). It should be emphasized that such a state of equilibrium is maintained even in 10% serum (Fig. 4), in which considerable degradation of vasopressin occurs. The proposed mechanism also explains that a slowly declining release replaces the constancy of liberation of vasopressin when either the release rate is enhanced by coloading with gelatin (12) or the load is in excess of the adsorption capacity of the polypropylene surface (200 μg of oxytocin, Fig. 3). In both cases, according to Langmuir absorption kinetics, the concentration of vasopressin in the void volume of the microporous polymer polypropylene will be relatively high and on release not replenished from the adsorbed stock. Consequently, the device empties itself quickly, which diminishes release rate more noticeably with time. A period of pseudo-zero-order release is finally reached regardless, *i.e.*, when the adsorbed stock is below the maximum capacity[13].

The collodion layer apparently prevents the rapid entry of many proteinaceous surfactants that could otherwise desorb vasopressin. Like vasopressin, oligopeptides from a medium will pass this membrane but in the opposite direction and will possibly enhance desorption. Comparing the curves in Figs. 1 and 2, this is not noticeable for 10% serum immersion, but *in vivo* (a wider variety of tissue compounds) this might be different (see below). The necessity of both microporous polymer and collodion for long-lasting release is shown when vasopressin preparations are implanted in Brattleboro rats in the absence of one or the other (Figs. 6B and E). Collodion itself, therefore, does not stipulate the constant hormone delivery, although it will undoubtedly influence release rate because of its thickness (6, 20, 21).

On the basis of this mechanism, the microporous polymer polypropylene–collodion preparation should prove applicable to any peptide, provided it sufficiently adsorbs to the polypropylene. This was true for oxytocin, lysine-vasopressin, and α-melanocyte-stimulating hormone, although constant release was not achieved for Met-enkephalin. The extent to which polypropylene adsorbs a peptide will depend on the hydrophobicity of the peptide (*i.e.*, its constituent amino acids). If polypropylene does not act properly for less hydrophobic peptides, perhaps appropriate internal surface coating or the use of other microporous polymers might give the required surface interaction.

Determination of Release Rate—Because dynamic equilibrium is reached in constant-volume incubations *in vitro*, the actual *in vivo* release should be higher. Assuming the body to be an infinite medium (outside vasopressin concentration is always zero), the release rate measured directly after the onset of *in vitro* incubation might be extrapolated and considered to be the release rate present *in vivo*. Such a calculation, on the basis of several curves as presented in Fig. 4, gives a release of 1–2% of the vasopressin load each day. This fits the radioactivity data on daily *in vivo* urine excretion (~1%) of iodinated vasopressin for 40 d. However, in general, *in vivo* rates cannot be derived easily from the release patterns *in vitro* and should be determined *in vivo*, *e.g.*, by implants with [^{3}H]vasopressin.

Vasopressin Release in the Brattleboro Rat—Long-term reduction of diabetes insipidus and reasonably constant excretion of vasopressin for several weeks were achieved by vasopressin implants (Fig. 7; note that compared with Fig. 2, vasopressin data are not expressed on a logarithmic scale). The higher excretion in the first few days was due to adaptation, probably at the level of the kidney (22, 23), since it was also seen with injected or minipump-driven vasopressin treatment (Fig. 8B, C). Assuming that ~1% vasopressin was extruded daily from the implant for 40 d, the 67% remaining in the stock should have a lower release rate. Since vasopressin urine excretion in rats is independent of infusion rate (24), the vasopressin excretion in Brattleboro rats should decline over time. The present data do not show such an effect. Only a twofold decrease occurs during the first 40 d for the 220-μg loaded implant and no decrease is observed for the 22-μg load (Fig. 7B, C). For high loads, *i.e.*, high release rates, the expected 1–2% of release per day is therefore an overestimation, probably due to the assumption of a zero subcutaneous concentration. In fact, a concentration gradient is to be expected around the vasopressin implant, which inhibits release (21) (it also could not account for the preparation acting over 100 d; Fig. 7C). In contrast to *in vitro* release (Fig. 4), the *in vivo* release indicated by daily urine vasopressin excretion appeared to be lowered with vasopressin tubing load (Table II). This additionally points to a protracted subcutaneous release of vasopressin. The rapidly decreasing release rate with the use of low-vasopressin contents of the microporous polymer tubing (Fig. 7A) is less easy to explain. Inactivation of the lower vasopressin stock may have a relatively higher threshold, or a tight encirclement of the tubing by connective tissue may be more effective in reducing the escape of vasopressin into the blood (25).

Daily injection of 100 mU of vasopressin tannate (*i.e.*, ~200 ng of vasopressin as a long-acting preparation) is shown to be the substitution dose of the Brattleboro rats (22). Normalization of diuresis ≥40 d, was brought about by implantation of a 22-μg vasopressin-loaded microporous polymer–collodion device (Table II) from which <220 ng/d is extruded. An approximately fivefold higher treatment (but supplied by an osmotic minipump) decreased urine production to near normal levels (Table II) (26). The present device, therefore, not only yielded a better alleviation of the diabetes insipidus (Table II), but also considerably prolonged the period with a more efficient use of vasopressin. Perhaps differences in release surface (entire surface of tubing *versus* small orifice of minipump) cause this effect, which could result in a different rate of uptake.

Concluding Remarks—The microporous polymer–collodion delivery module appears to be biocompatible and can be used as an implant in adult rats. Its use requires a simple operation and treatment is easily terminated. When the commercially available osmotic minipump is too large for implantation, the size of the microporous polymer delivery system allows peptide treatment of young and immature laboratory rats. The potential of the present device, moreover, is underlined by the possibility of prenatal application with smaller-sized microporous polymer tubing. The release properties of this 0.7-mm diameter tubing have been examined only in pilot studies, but did give a lasting release for vasopressin *in vitro* as well[14].

The adsorption-based mechanism of the constant delivery makes the microporous polymer technique a novice in the field of polymer monolith or membrane-controlled drug delivery systems (5, 6, 21). Whenever adsorption can be introduced on the typical microporous structure of microporous polymer and protection against the rapid desorption from outside compounds is possible, the device will provide a constant and lasting release for other peptides and possibly for other physiological compounds and pharmaceuticals as well.

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Sustained-Release Characteristics of a New Implantable Formulation of Disulfiram

MICHAEL PHILLIPS *§x **and JOSEPH D. GRESSER** ‡¶

Received November 7, 1983, from the **Division of Internal Medicine, Georgetown University Hospital, Washington, DC 20007* and *‡Dynatech Research and Development Company, Cambridge, MA 02139.* Accepted for publication March 1, 1984. Present addresses: §Division of General Medicine and Clinical Pharmacology, The Chicago Medical School, North Chicago, IL 60064 and ¶Key Pharmaceuticals, Inc., Miami Beach, FL 33169.

Abstract □ The object of this study was to evaluate the sustained-release characteristics of a new formulation of disulfiram. Solid rods (500 mg) made of a composite of 80% poly(glycolic-co-L-lactic acid) and 20% ^{14}C-labeled disulfiram were implanted subcutaneously in five Wistar CD-1 rats; a control group received 100 mg of ^{14}C-labeled disulfiram subcutaneously. Excretion of radiolabeled material in the urine and feces was monitored for 88 d. Sustained mobilization of drug was observed in the copolymer–disulfiram implant group, reaching a peak value 30 d after implantation. The control group exhibited first-order kinetics of drug mobilization. At necropsy, there was no encapsulation of the residual rods. The copolymer–disulfiram composite performed as a true sustained-release system, and improved formulations may have clinical applications in the treatment of alcoholic humans.

Keyphrases □ Disulfiram—sustained-release implantable formulation, rats, poly(glycolic-co-L-lactic acid) □ Sustained-release formulations—implantable disulfiram, poly(glycolic-co-L-lactic acid), rats

Disulfiram is widely prescribed to discourage alcoholics from drinking alcohol, since the two drugs interact to produce a subjectively unpleasant experience characterized by facial flushing, nausea, tachycardia, and hypotension (1–4). The effectiveness of disulfiram as a treatment for alcoholism is severely limited by the willingness of patients to take the drug every day; many stop taking their tablets so that they might resume drinking alcohol as soon as the effects have worn off (5). Frequent failures of treatment with the orally administered drug have stimulated interest in parenteral therapy with subcutaneously implanted disulfiram tablets, but numerous studies during the past 25 years have demonstrated that these implants have miniscule pharmacological effects, possibly due to their poor bioavailability (6–8).

However, animal studies have demonstrated that disulfiram can be rapidly mobilized from a subcutaneous site, provided that the drug is injected in an appropriate vehicle, *e.g.*, suspended in arachis oil (9) or dissolved in polyethylene glycol (10). These findings suggest that it might be possible to prepare a sustained-release disulfiram implant with a true pharmacological effect. Ideally, such a formulation would combine disulfiram with a vehicle which would deliver the drug into the circulatory system at a steady rate for several weeks or months at a time and be free of any significant local or systemic toxicity. A vehicle which appears to offer these features is a new biodegradable polymer, poly(glycolic-co-L-lactic acid) (PLGA). When implanted subcutaneously, the copolymer appears to degrade slowly into its parent monomers, lactic acid and glycolic acid, while continuously releasing any bound drug at a steady rate. *In vivo* studies of PLGA combined with contraceptives, narcotic antagonists, and antimalarials have shown that these implants can deliver the drug continuously into the circulatory system for several months at a time (11). We describe a study of the sustained-release characteristics of a new formulation of disulfiram combined with the copolymer, which was undertaken to investigate the feasibility of using such a preparation in the treatment of alcoholic humans.

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