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The use of [^{3}H]vasopressin for in-vivo studies of controlled delivery from an Accurel/collodion device in the Brattleboro rat

J. KRUISBRINK* AND G. J. BOER

Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam ZO, The Netherlands

Accurel polypropylene/collodion controlled drug-delivery devices containing 22 μg [^{3}H]-vasopressin (VP) were implanted s.c. in VP-deficient Brattleboro rats. This caused a decrease in their urine production for at least 50 days, at which time the Accurel device was removed. Release of VP was followed by measurements of tritium radioactivity and by radioimmunoassay of VP in the urine. These parameters showed a constant pattern during the whole period. After re-implantation of the Accurel devices in another group of Brattleboro rats, release of VP continued to give the same level of urine production as during the last period of the first implantation. It is concluded that release of VP in-vivo is not influenced by encapsulation of the Accurel polymer by connective tissue, or that adaptation of the kidney adds to the maintenance of the urine production at this low level. In-vivo release rate is calculated to be about 1% of the original load each day. The in-vitro release in a flow cell system appeared to produce the same release rate which indicates that these data have a predictive value for the in-vivo situation.

A pseudo zero-order release of vasopressin (VP) is realized when an Accurel polypropylene/collodion device loaded with VP is incubated in-vitro in a proteinaceous medium (Boer et al 1983; Kruisbrink & Boer 1984). When implanted subcutaneously in a rat, such a device loaded with 22 μg VP normalized diuresis of homozygous VP-deficient Brattleboro rats for 60 days. The exact in-vivo release rates, however, cannot be derived easily from the release patterns in-vitro. Release rates from polymer devices in-vivo can be perturbed either by the presence of unstirred layers adjacent to the device or by encapsulation of the device by fibrous tissue (Baker & Lonsdale 1974). In addition, changes in the dosage rate might not be detected since they can give rise to the same, maximal, biological effect when the rate remains within the saturated part of the dose-response curve. Also, for the Brattleboro rat the kidney function might adapt to a long-term treatment of VP (Bankir et al 1985).

In the present study, therefore, the steady state of the release rate was determined in-vivo by following the appearance of tritium radioactivity as well as VP immunoreactivity in the urine after implantation of a [^{3}H]VP-containing Accurel/collodion device in a Brattleboro rat. The in-vivo release rate was compared with the in-vitro release rate as found in a continuous flow system. The possible effect of encapsulation of the device on the in-vivo release rate was investigated by removal of the device after seven weeks and re-implantation in a second Brattleboro rat.

* Correspondence.

MATERIALS AND METHODS

Materials

Animals. Male Brattleboro (di/di) rats (65 days old; ca 220 g), congenitally deficient for brain VP, were obtained from Brattleboro rat breeding; the parents were obtained from CPB/TNO (Zeist, The Netherlands). The animals were kept under standard conditions in metabolism cages throughout the experiment.

Chemicals. Vasopressin (VP) (grade VIII, lot no. 92F-03481, 403 u mg^{-1}) was obtained from Sigma (St Louis, USA). [^{3}H]VP (specific activity: 45·3 Ci mmol^{-1}) was obtained from NEN (Boston, USA).

Methods

Accurel technique. The Accurel/collodion device was prepared as described previously (Boer et al 1983; Kruisbrink & Boer 1984). In the present study, a 2 cm piece of Accurel polypropylene tubing (P 78/15/2) was lumen-filled with 7·5 μl aqueous VP-solution (3 μg μl^{-1}), heat-sealed at both ends and coated with collodion. The VP solution was tracered with [^{3}H]VP (final specific activity ca 170 mCi mmol^{-1}).

Implantation of vasopressin-loaded Accurel polymer. After a control period of three days in which the mutant rats showed diabetes insipidus (DI), the animals received, subcutaneously, under light ether anaesthesia a VP/Accurel/collodion implant. The effect of VP release was followed in three animals by measurements of daily urine production and aliquots (1 ml) were collected for tritium counting and for radioimmunoassay of VP. Radioactivity in the urine was counted after addition of 250 µl 1 M HCl to reduce quenching in the 10 ml of Insta-gel (Packard) scintillation fluid. Before radioimmunoassay, the urine samples were stored at −20 °C and VP was extracted with Vycor glass powder before assay (Dogterom et al 1977).

Body weights were monitored every 3–4 days. After 7 weeks the implant was removed, again under ether anaesthesia, and thereafter incubated under in-vitro conditions for 7 days, i.e. daily immersions in 1 ml volumes of 0·5% albumin at 37 °C (Boer et al 1983; Kruisbrink & Boer 1984). Subsequently, the VP/Accurel/collodion devices were re-implanted in three other male Brattleboro rats, who also had a control period of three days in metabolism cages. After this in-vivo re-use of the preparation for 10 days, again the implant was removed and this was followed by an in-vitro release period of another 7 days. The in-vitro samples were used for tritium radioactivity measurements as well as for radioimmunoassay of the VP.

Flow cell system for in-vitro release of vasopressin. The release of VP from the Accurel delivery device was also followed by in-vitro perfusion at 37 °C in 0·5% bovine serum albumin containing 0·01% sodium azide using a continuous flow system. The flow cell system consisted of cylindrical holes (1 cm height, 1·5 ml volume) which were drilled into a Perspex block with separate metal in- and outlets (i.d. 1·0 and 0·5 mm) in the screw-cap and bottom, respectively. The flow through the system was 300 µl h^{-1} (Watson-Marlow pump, 202 U) and fractions were collected every 4 h in a fraction collector. Release of VP was followed by counting the tritium radioactivity.

Statistics. Student's *t*-tests were used to determine significance of differences, with $P < 0{\cdot}05$ as the level of significance.

RESULTS

In-vivo release in the Brattleboro rat

Immediately after implantation of the VP/Accurel/collodion device (22 µg VP/8·25 × 10^6 $dmin^{-1}$) in three Brattleboro rats, diuresis decreased from ca 70 to 10–20 ml/100 g body weight/day for a period of seven weeks (Fig. 1). However, when in this period the first week is compared with the last week, a small but significant increase is observed in diuresis as well as in tritium excretion in the urine (Table 1). Daily urine VP content remained constant (3·5 ng VP/day; s.e.m. = 0·2) as measured daily during the first two weeks and thereafter with 2-day intervals (cf. Fig. 1C and Table 1). Using the data on the specific radioactivity of the tracered VP in the implant (375 $dmin^{-1}$ ng^{-1}; see above), this amount of VP should give a tritium excretion of 1310 $dmin^{-1}$ day^{-1}. Over the seven-week period, a total of 1·34 × 10^6 $dmin^{-1}$ tritium was excreted with the urine, which therefore gives a higher daily average of 27 200 $dmin^{-1}$ (s.e.m. = 660). Consequently, it can be calculated that an average of 4·8% of the radioactivity excreted in the urine is due to undecomposed VP.

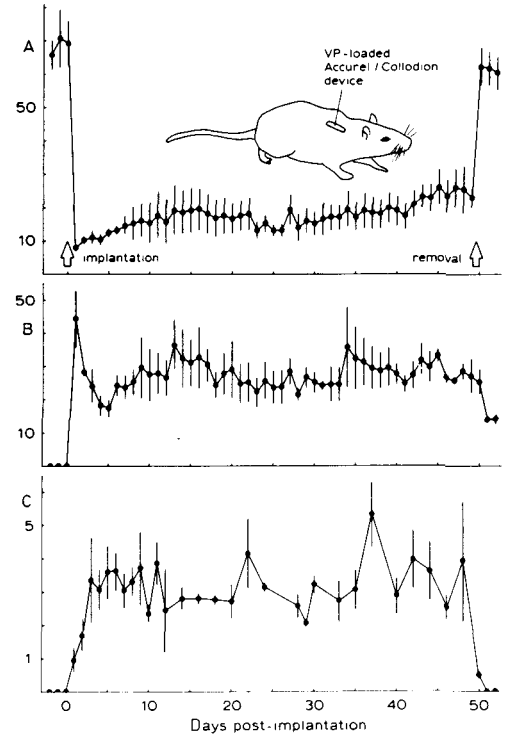

FIG. 1. Replacement therapy of vasopressin (VP) in the VP-deficient Brattleboro rat using a 22 µg [^{3}H]VP-loaded Accurel/collodion subcutaneous implant. Data are average ± s.e.m. (n = 3). A, urine production (ml/100 g day^{-1}); B, ^{3}H-urine excretion ($dmin^{-1}$ × 10^{-3} day^{-1}); C, VP urine excretion (ng day^{-1}).

Table 1. Comparison of average urine excretion data (± s.e.m.) of first and second use of a [^{3}H]vasopressin-loaded Accurel implant in the Brattleboro rat.

	Urine production (ml/100 g day^{-1})	Tritium excretion (dmin^{-1} day^{-1})	VP excretion (ng day^{-1})
1st week 1st implantation	13 ± 0·8*	23 000 ± 1600[a]	3·5 ± 0·3
Last week 1st implantation	25 ± 0·5	29 000 ± 1090*	3·5 ± 0·5
1st week 2nd implantation	24 ± 1·0	19 500 ± 930	1·8 ± 0·3*

[a] The first two days of implantation were omitted since impurities of the [^{3}H]VP were then released.
* Statistically different from the other two periods.

Upon removal of the device, diuresis returned to pre-operative values (Fig. 1). VP excretion in the urine also immediately returned to undetectable levels though the tritium excretion remained at an intermediate level for at least several days (14 250 ± 700 dmin^{-1} day^{-1}; cf. Table 1). Re-implantation of the Accurel devices into three other Brattleboro rats after an in-vitro release period of one week, again immediately decreased urine production to an average level of 23 ml/100 g body weight/day (s.e.m. = 1·7) for 10 days (Fig. 2), a level fully comparable with the last week average of the first implantation period (Table 1). Tritium excretion data were similar in the first week of both implantation periods, but VP excretion was 51% lower (1·8 ng; Table 1). Average daily tritium excretion in the urine was now 18 700 dmin^{-1} (s.e.m. = 1024), which means that in the urine 3·6% of the radioactivity is undecomposed VP.

From day 50 to 56 (i.e. after the first implantation) and from day 69 to 76 (i.e., after the second implantation), the Accurel device, incubated in-vitro, showed a daily constant release of tritium radioactivity and radioimmunoassay detectable VP (Fig. 3). The average amount of tritium released amounted to 0·4 and 0·2%, respectively, of the original tracer load of the device.

After the entire 76 days, radioactivity was still present in the devices and could be recovered by incubation in methanol containing 0·01% triton-X-100. An average of 3 × 10^6 dmin^{-1} could still be released, i.e. 36% of the original load. When this amount, as well as the amounts released in-vitro, are subtracted from the total amount of tracer originally loaded in the device (gives 5 × 10^6 dmin^{-1}), and this figure is divided by the number of days of subcutaneous application (58 days), the average daily release rate is 84 000 dmin^{-1}, i.e., ca 1% of the original load each day (for the 22 µg-loaded device, this means a release of ca 220 ng day^{-1}).

In-vitro release in a continuous flow system

In-vitro release rate of VP is dependent on the flow rate and albumin concentration of the medium, but reaches its maximum in a 0·5% albumin solution at a flow rate of 300 µl h^{-1} (unpublished results). Under these conditions a 22 µg-filled VP/Accurel/collodion device released [^{3}H]VP at an average rate of 0·7% of its load each day for at least 25 days (Fig. 4).

DISCUSSION

Release pattern upon implantation in the Brattleboro rat

From our experiments it can be concluded that the in-vivo release of VP from a VP/Accurel/collodion device is long-lasting over a period of several weeks. Since the three parameters tested showed only little change over time (Fig. 1) and no 'burst' effects were noticeable, a pseudo zero order release rate can be assumed. These data confirm our previous findings (Boer et al 1983; Kruisbrink & Boer 1984). After re-implantation of the Accurel device into another series of Brattleboro rats, the same antidiuretic

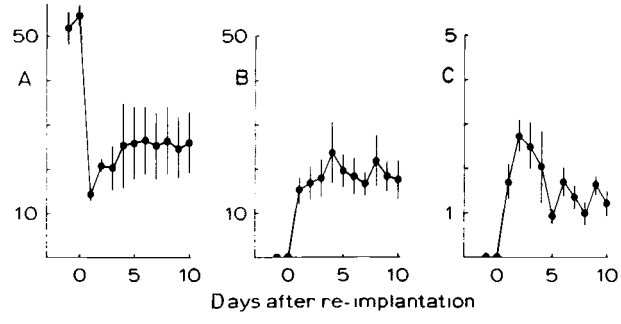

FIG. 2. Replacement therapy of vasopressin (VP) in the VP-deficient Brattleboro rats. Re-use of the implants of Fig. 1 in other rats. Data are average ± s.e.m. (n = 3). A, B and C as in Fig. 1.

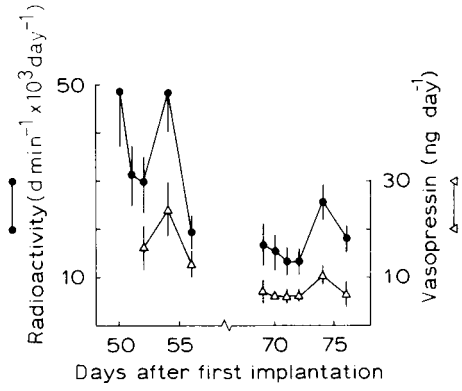

FIG. 3. Two periods of in-vitro release of tritium and vasopressin (VP) from a [^{3}H]VP-loaded Accurel/collodion device immersed in a daily renewed 1 ml 0·5% albumin solution, in between and after the subcutaneous use in Brattleboro rats. Data are average ± s.e.m. (n = 3).

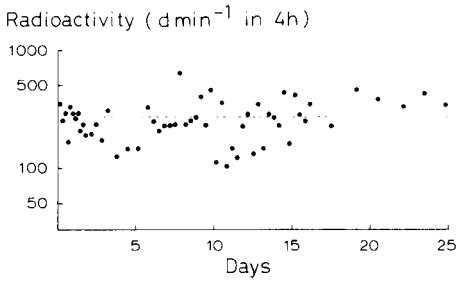

FIG. 4. Tritium radioactivity released from a [^{3}H]vasopressin (VP)-loaded Accurel/collodion device using a flow cell system connected with a fraction collector set on 4 h intervals. The device was loaded with 22 μg VP tracered with 2·36 × 10^5 dmin^{-1}. The average release rate during 25 days was 270 dmin^{-1}/4 h, i.e. 0·7% (154 ng) of the load each day.

effect is seen as at the end of the first implantation period, which indicates that the release rate in-vivo is not influenced by encapsulation with connective tissue, nor that long-term adaptation of the kidney adds to the maintenance of the urine production at a low level.

However, apparently no complete zero-order release kinetics are present, since the 82% reduction in the polyurea of the Brattleboro rat diminished slowly to reach a 64% reduction at the end of the 7-week period. This might be caused by a reduced release of VP from the subcutaneous implant. Previously we reported that the release rate from the device is related to its load (Boer & Kruisbrink 1984; Boer et al 1984; Kruisbrink & Boer 1984). Following an in-vivo fractional release of approximately 1% each day (see also below), a slowly declining release rate would be expected. Nevertheless, the daily VP excretion via the urine remained constant (Table 1). These conflicting results can only be explained by assuming a gradual increase in clearance of VP at the kidney level. On the other hand, recent data from Cheng et al (1982) showed that VP content of daily urine was not a sensitive marker for small changes in plasma VP levels.

Less surprising is the 26% increase in tritium excretion. All tissues of the rat contain considerable amounts of tritium after the 7 weeks of implantation (not shown), which demonstrates that the tracer is incorporated in all kinds of compounds and metabolites of the body. This will therefore give rise to a second stream of tritium excretion not directly related to [^{3}H]VP release from the Accurel implant. The average amount of tritium still excreted in the urine after removal of the Accurel/collodion device (14 250 dmin^{-1}), as well as the difference between tritium excretion data of the last week average of the first in-vivo period, and the first week average of the second in-vivo period (9500 dmin^{-1}; cf. Table 1), gives an indication of the contribution from this pool to the daily tritium excretion after seven weeks. There is a 15% difference, though not statistically significant, between the tritium excretion at the onset of both in-vivo implantation periods (Table 1). In our opinion, this indicates once more that the release rate of VP changed only slightly over a period of weeks.

The excretion of immunoreactive VP in the urine is constant during both implantation periods but the amount excreted in the second period is 51% lower. Cheng et al (1982), using osmotic minipumps for subcutaneous release, found a linear relation between the infusion rate of VP and urine production of the Brattleboro rat, but no correlation between infusion rate and urinary excretion of VP. Therefore, the different values found in the two groups of animals during the first and second implantation are very likely due to variation between the groups of animals.

The percentage of tritium excreted in the urine that comes from intact VP during the first and last week of the first implantation period are 5·6 and 4·6%, respectively (whole period average 4·8%). When the figure for the last week is corrected for the contribution of the second stream of tritium (see above), it is found to be 6·9% instead. In the first week of the second implantation, 3·6% of the tritium in the urine consisted of immunoreactive VP. These data, therefore, do not change very much, although they are based on the assumption that no breakdown of VP exists inside the Accurel collodion implant.

The present figures are on the low side of the rather wide range that is given in the literature: 6–37% (Lauson 1974).

Comparison of in-vivo with in-vitro release rates

So far, an easy direct comparison of in-vitro and in-vivo release rates has not been possible. The mechanism of action of the Accurel device is probably based on high adsorption of the peptide onto the large internal surface area of the polypropylene microporous matrix and outward diffusion by the low concentration of peptide in the water-filled void volume of the tubing wall (Boer & Kruisbrink 1984; Kruisbrink & Boer 1984). In-vitro release in a small volume therefore slows down and stops completely when the equilibrium of concentrations in- and outside the Accurel matrix is reached (Kruisbrink & Boer 1984). This is the reason why the in-vitro data of Fig. 3 do not give actual release rates. Based on the assumption that the in-vivo situation is comparable to 'infinite sink' conditions, the in-vivo daily release rate has been estimated by extrapolation to 24 h of the initial release in-vitro under these conditions (Kruisbrink & Boer 1984). In this way, in-vivo release rates became about 1–2% of the load each day. This is somewhat higher than the 1% daily release as calculated from the in-vivo data presented here, as well as from those in a pilot study with [^{125}I]VP excretion in-vivo (Kruisbrink & Boer 1984). Measurements in a continuous flow system should give a better estimation of the maximum release rate. For the present device applied in-vivo, in-vitro release rate per 4 h intervals varied around a constant level of about 0·7% fractional release rate for 25 days. Under these 'infinite sink' conditions a better approximation to the in-vivo situation was obtained, which supports the idea that this flow system can be used to approximate actual in-vivo release rates.

Replacement therapy in the Brattleboro rat

A curious observation using the present technique for supplementation of VP in the Brattleboro di/di rat is the fact that much less VP seems to be acquired each day than when an Alzet osmo-minipump is used as described elsewhere (Cheng et al 1982; Kruisbrink & Boer 1984). With the latter technique, threshold VP release for initiation of antidiuresis was between 20 and 50 ng day^{-1}, whereas very high levels of 3000 ng day^{-1} were necessary to normalize diuresis (Cheng et al 1982). Previously we showed that a rate of 1000 ng day^{-1} from a minipump (i.e. a load of 22 μg VP) only partially suppressed the polyurea (Kruisbrink & Boer 1984). On the other hand, in our previous studies (Kruisbrink & Boer 1984) as well as in this study, the 22 μg VP-loaded Accurel/collodion preparation fully corrected the diuresis for the three-week period, the maximal period in which the minipump would have acted. Moreover, it acts by means of a release rate of only 220 ng day^{-1}. Perhaps the differences in release surface area (entire surface of tubing vs small orifice of minipump) cause this effect, since these may have affected the rate of uptake by the body. For subcutaneous application therefore, the present technique, when compared with the minipump, is superior in duration of action and efficiency of dosage as well as costs. However, the present technique can only be used if the compound to be released strongly adsorbs to a polypropylene surface (Kruisbrink & Boer 1984) and when there is no need for very high dosage rates.

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