Table 2. Organ weights and serum hormone concentrations of male rats (n = 7) bearing subcutaneous implants with or without 10% LH–RH or TRH over five days and after reimplantation in second recipient for five days.

| | Treatment with s.c. implants | Organ weights Testes (g) | Seminal vesicles (mg) | Ventral prostate (mg) | Musculus levator ani (mg) | Thyroid (mg) |
|---|---|---|---|---|---|---|
| 1st Implantation | Empty | 2·5 ± 0·06 | 177 ± 14 | 192 ± 13 | 165 ± 9 ns | 14·1 ± 0·8 |
| | 10% LH–RH | 1·9 ± 0·09*** | 183 ± 10 ns | 193 ± 6 | 174 ± 7 ns | — |
| | 10% TRH | 2·5 ± 0·08 ns | 152 ± 11 ns | 158 ± 8* | 148 ± 5 | 17·1 ± 0·6* |
| Re-implantation | Empty | 2·3 ± 0·04 | 92 ± 11 ns | 123 ± 9 | 121 ± 7 | — |
| | 10% LH–RH | 1·7 ± 0·1*** | 98 ± 9 ns | 149 ± 8* | 131 ± 5 ns | — |
| | 10% TRH | 2·3 ± 0·06 ns | 93 ± 9 ns | 140 ± 16 ns | 130 ± 10 ns | — |

| | | Serum hormone concentrations (ng ml$^{-1}$) Day 3 | | Day 5 | | | | |
|---|---|---|---|---|---|---|---|---|
| | | LH | FSH | LH | FSH | PRL | TSH | LH–RH |
| 1st Implantation | Empty | 11 ± 2 | 410 ± 27 | 37 ± 5 | 432 ± 28 | 55 ± 7 | 264 ± 57 | 0·42 ± 0·02 |
| | 10% LH–RH | 112 ± 10*** | 376 ± 42 ns | 83 ± 10** | 293 ± 16** | 45 ± 6 ns | 247 ± 32 ns | 8·22 ± 2·71** |
| | 10% TRH | 14 ± 2 ns | 502 ± 62 ns | 27 ± 5 ns | 423 ± 24 ns | 66 ± 11 ns | 479 ± 124 ns | — |
| Re-implantation | Empty | 18 ± 3 | 436 ± 31 | 21 ± 5 | 466 ± 27 | 44 ± 9 | 234 ± 45 | — |
| | 10% LH–RH | 113 ± 12*** | 433 ± 27 ns | 74 ± 8*** | 350 ± 21** | 80 ± 14 ns | 289 ± 17 ns | — |
| | 10% TRH | 38 ± 5** | 524 ± 13* | 30 ± 6 ns | 523 ± 12 ns | 42 ± 5 ns | 668 ± 80** | — |

Results are mean values ± s.e.m. (ns = statistically not significant, *$P<0.05$, **$P<0.01$, ***$P<0.001$).

of chemical breakdown (D. Gillessen, personal communication). The results obtained with LH–RH agree well with those of other groups which demonstrated similar effects with LH–RH or more potent analogues (Corbin & Beattie 1975; Auclair et al 1977) after repeated injections. They explained their findings by postulating loss of testicular LH/hCG and prolactin receptors. A marked reduction in testicular weights and circulating testosterone concentrations occurs, possibly in consequence of this effect. The testosterone remaining is nonetheless obviously adequate to prevent atrophy of the accessory sex organs in our experiments. None of the other investigators observed a decrease in circulating FSH concentrations. Whether the reduced FSH concentrations we found are the result of increased negative feedback or reduced hypophysial synthesis is unclear. The new method of peptide administration we describe has the advantage over established techniques of providing a presumably essentially constant low-dose supply of the substance implanted, thus simulating the process of basal secretion.

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# The absorption of saccharin from the rat urinary bladder

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Recently it has been reported (Colburn 1978) that saccharin is absorbed significantly from the rat urinary bladder, since only 43–80% of a dose was recovered in the bladder 2 h after instillation of [$^{35}$S] saccharin via a bladder cannula. This observation is of interest since the bladder is the organ in which tumours were detected during long-term feeding studies. Furthermore, it has wider implications since the resulting plasma concentrations of saccharin, which were constant between 5 and 120 min, were reported to be sufficient to alter the pharmacokinetics of this highly ionic, water soluble compound. In these studies the ureters were not reported to have been ligated and since the renal clearance of saccharin is high (Goldstein et al 1978) it is likely that considerable recycling back into the bladder may have occurred and hence the true extent of absorption under-estimated. We have undertaken a series of experiments in order to elucidate the extent of saccharin reabsorption from the bladder.

To investigate the extent of recycling, saccharin absorption was studied in rats in which a cannula was inserted through the bladder wall and tied securely to

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prevent leakage into the peritoneal cavity. Both ureters were cannulated for collection of urine and ligated below the cannulae. [$^3$H] Saccharin in water (500 $\mu$Ci; 0·1 ml; 100 mg kg$^{-1}$) was introduced via the bladder cannula and urine and plasma samples (from an external jugular cannula) were collected at 15 min intervals and the $^3$H content determined by liquid scintillation counting. Under these circumstances the plasma levels (12 000 ng ml$^{-1}$ for 100 mg kg$^{-1}$) were similar to those reported previously but decreased during the 2 h period (Table 1). The recovery from the bladder was only 33% of the dose but the urine collected during the 2 h contained 12% of the dose. This suggests that recycling would have occurred if the ureters were intact, which would explain the constant plasma concentrations reported previously and the higher recovery in the bladder (59% of the dose).

To investigate the permeability of the bladder in the absence of cannulation we have studied the absorption of saccharin introduced via a cannula inserted into the lower part of one ureter. Retrograde cannulae were inserted into both ureters for urine collection and the lower part of the second ureter was ligated to prevent leakage. During this operation the bladder was manipulated as little as possible and no attempt was made to remove any urine present (0·2–1·1 ml). The [$^3$H] saccharin (0·03–100 mg kg$^{-1}$ in 0·1 ml water) was instilled into the bladder via the ureter and the cannula volume displaced by isotonic saline (0·05 ml). Urine and plasma samples were collected and analysed as in the previous experiments. Under these conditions the plasma concentrations (Table 1) were approximately 25 times lower than those reported by Colburn (1978), and contained an average of only 0·0005% of the dose ml$^{-1}$ (392 ng ml$^{-1}$ after 100 mg kg$^{-1}$) compared with his values of 0·01–0·04% ml$^{-1}$ (29 000 ng ml$^{-1}$ after 100 mg kg$^{-1}$) in the 6 animals studied. This markedly reduced absorption was reflected in an increased recovery in the bladder (91% of dose) and a diminished elimination via the ureters (0·27% of dose). There was no evidence to suggest an increased rate of absorption at higher doses as reported by Colburn (1978). In addition, feeding 5% w/w sodium saccharin diet for 3 months, did not alter the absorption of [$^3$H] saccharin instilled into the bladder.

Interference with plasma pharmacokinetic measurements by saccharin reabsorbed from the bladder, as suggested by Colburn (1978) was not detected in normal rats. The elimination half-lives and plasma clearance values were the same in rats with intact bladders, in which excreted saccharin was available for reabsorption, and in rats in which the bladders were cannulated but the contents removed and rinsed out every 15 min. However, the half-life was significantly increased and the clearance decreased if the bladder was cannulated but not emptied and rinsed during the 2 h study (Table 2).

Thus, our results show that saccharin is absorbed very slowly from the normal rat bladder, which is in agreement with the studies of Sargent et al (1979) who found that the absorption of a series of n-alkyl carbamates from the female rat bladder was limited by their hydrophilicity. However, cannulation of the bladder

Table 1. The concentrations of [$^3$H] saccharin in the plasma and urine of rats after instillation into the urinary bladder. Adult male Charles Rivers derived Sprague Dawley rats (300–400 g) were anaesthetized with pentobarbitone and both ureters cannulated for the collection of urine. One ureter was cannulated for introduction of the dose into the bladder and the other was ligated. Blood and urine samples were collected over 2 h after which the bladder was emptied and rinsed with saline (3 × 1 ml). (n=no. of rats).

| | Dose (mg kg$^{-1}$): | | | | |
|---|---|---|---|---|---|
| Time (min) | 0·03 (n = 4) | 0·03† (n = 3) | 10 (n=3) | 100 (n = 3) | 100* (n = 3) |
| | [$^3$H] saccharin concn in plasma (ng ml$^{-1}$) | | | | |
| 15 | 0·37 (0·24) | 0·10 (0·08) | 53 (12) | 445 (27) | 15 700 (5300) |
| 30 | 0·25 (0·10) | 0·09 (0·06) | 52 (37) | 450 (234) | 15 300 (5300) |
| 45 | 0·31 (0·19) | 0·08 (0·05) | 56 (49) | 370 (59) | 13 000 (4800) |
| 60 | 0·23 (0·12) | 0·13 (0·11) | 59 (57) | 316 (40) | 11 300 (6000) |
| 75 | 0·20 (0·11) | 0·07 (0·03) | 52 (47) | 324 (60) | 10 300 (6100) |
| 90 | 0·22 (0·12) | 0·08 (0·06) | 57 (41) | 375 (171) | 9700 (5500) |
| 105 | 0·23 (0·07) | 0·08 (0·04) | 65 (37) | 444 (195) | 10 100 (5700) |
| 120 | 0·19 (0·10) | 0·07 (0·03) | 51 (36) | 408 (140) | 10 800 (6800) |
| Time (min) | % dose in urine | | | | |
| 15 | 0·00 (0·00) | 0·04 (0·03) | 0·00 (0·00) | 0·02 (0·02) | 0·59 (0·52) |
| 30 | 0·05 (0·04) | 0·04 (0·03) | 0·01 (0·02) | 0·07 (0·03) | 2·47 (1·21) |
| 45 | 0·04 (0·04) | 0·03 (0·02) | 0·04 (0·02) | 0·05 (0·03) | 1·75 (0·70) |
| 60 | 0·03 (0·03) | 0·02 (0·01) | 0·04 (0·04) | 0·06 (0·03) | 2·34 (1·11) |
| 75 | 0·03 (0·03) | 0·01 (0·01) | 0·05 (0·07) | 0·02 (0·01) | 1·66 (0·65) |
| 90 | 0·03 (0·04) | 0·01 (0·01) | 0·06 (0·06) | 0·03 (0·01) | 1·21 (0·37) |
| 105 | 0·03 (0·03) | 0·01 (0·01) | 0·06 (0·06) | 0·03 (0·03) | 1·18 (0·53) |
| 120 | 0·03 (0·04) | 0·01 (0·01) | 0·08 (0·10) | 0·05 (0·04) | 1·25 (1·03) |
| 0–120 total | 0·24 (0·25) | 0·17 (0·10) | 0·34 (0·35) | 0·32 (0·12) | 12·45 (3·29) |
| | Recovered in bladder | | | | |
| | 89·5 (2·1) | 95·9 (8·2) | 86·4 (16·9) | 91·2 (3·7) | 32·8 (31·3) |

The results are the mean with standard deviation in parentheses.

† Animals fed 5% w/w sodium saccharin diet for 3 months before study.

* Animals in which the dose was instilled via a bladder cannula. Both ureters were ligated distal to the urine collection cannulae.

Table 2. The pharmacokinetics of i.v.[$^3$H]saccharin (100 mg kg$^{-1}$; 100 $\mu$Ci/rat) in pentobarbitone anaesthetized normal rats by the analysis of blood samples (0·2 ml) collected at 15 min intervals. No urine was passed in the 2 h of sampling. Measurements were also made in rats with bladder cannulae in which the bladder was either left for 2 h (unrinsed) or emptied and rinsed every 15 min (rinsed).

| | Normal | Bladder cannulated rinsed | unrinsed |
|---|---|---|---|
| No. of animals: | 6 | 8 | 5 |
| Half-life (min) | 22·4 (3·5) | 22·4 (3·2) | 31·8 (8·2)* |
| Apparent volume of distribution (ml kg$^{-1}$) | 412 (132) | 408 (141) | 378 (89) |
| Plasma clearance (ml min$^{-1}$ kg$^{-1}$) | 13·2 (5·3) | 13·0 (5·6) | 8·6 (2·8) |

The results are the mean (standard deviation).
* $P<0·05$ compared with normals.

markedly increased its permeability and under such circumstances sufficient absorption to interfere with plasma pharmacokinetics may result, if the urine is not removed regularly. In this context we noted that even manipulation of the bladder with forceps or gentle palpation in an attempt to expel urine resulted in an increase in apparent permeability. Our results emphasize the care that is necessary in the interpretation of quantitative biological data obtained during experiments in which the normal physiological state of an organ is altered by the investigation.

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# LETTERS TO THE EDITOR

## Which pharmacological action of baclofen matters most?

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Kerwin & Pycock (1978) have shown that very high concentrations of baclofen, the $\beta$-(p-chlorphenyl) derivative of $\gamma$-aminobutyric acid (GABA), increase the radioactive content of superfusates of rat globus pallidus slices prelabelled with $^3$H-GABA in vitro, and suggest that this compound may act as a GABA-releasing agent in vivo. While another recent study would support this view (Roberts et al 1978), earlier work has indicated that lower, and perhaps more physiologically relevant concentrations of baclofen may inhibit the release of GABA (Potashner 1978). Also, there seems to be some debate as to the pharmacological relevance of GABA-releasing effects seen with concentrations of baclofen greater than 100 $\mu$M. For example, Kerwin & Pycock (1978) find baclofen to be inactive at 100 $\mu$M and suggest that significant effects seen with 0·3–1·0 mM baclofen may be important, while Olsen et al (1978) consider that the absence of any significant effect of baclofen at 100 $\mu$M indicates the inactivity of this compound.

We feel that recent studies on the ability of baclofen to displace $^3$H-GABA specifically bound to brain membrane preparations in vitro may be more relevant to the GABA mimetic action of this compound. Studies in this area are contentious, but both Olsen et al (1978) and ourselves (Waddington & Cross 1979) have found (±)-baclofen to displace $^3$H-GABA binding with IC50's of 55 and 40 $\mu$M respectively. These concentrations of baclofen are considerably smaller than those reported by Kerwin & Pycock (1978) to enhance GABA release (0·3–1·0 mM). While other studies have failed to observe such low IC50's for displacement of $^3$H-GABA binding by baclofen (Roberts et al 1978; Galli et al 1979; Lloyd & Dreksler 1979), they do not report if there was significant displacement of binding at higher concentrations. This may be important as

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