Release of oligopeptides from silicone rubber implants in rats over periods exceeding ten days

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Experimental studies and clinical development of physiologically active peptides have been hampered or even prevented by the fact that circulating peptides are rapidly degraded. Some success has been achieved by chemical alterations of such substances which extended their duration of action by increasing resistance to enzymatic attack. We have shown that two oligopeptides of hypothalamic origin are released from silicone rubber over a period of several days after subcutaneous implantation in rats; we assume that this principle should be applicable to similar compounds such as ACTH fragments and enkephalins.

For our studies we mixed lyophilized luteinizing hormone-releasing hormone (LH–RH)†, a decapeptide, and thyrotrophin-releasing hormone (TRH)†, a tripeptide, with Medical Elastomer 382 (Dow Corning) on a hollow-ground slide. After polymerization initiated by the addition of the catalyst supplied, the flat disk was cut into strips of almost identical size and weight (3 mg peptide in 30 mg plastic). Male Füllinsdorf albino rats (Ibm: RORO$_f$SPF) were kept in rooms under controlled conditions (22 ± 1 °C; lights on between 05·00 and 19·00 h; relative humidity 50%; 16 air changes h^{-1}). Empty and peptide-impregnated elastomer strips were implanted subcutaneously under ether anaesthesia by fixing to skeletal muscle with silk thread through a small mid-dorsal incision.

At various times after implantation, blood was collected either from the jugular vein under ether anaesthesia or, after decapitation, from the trunk. The serum obtained after centrifugation was kept deep-frozen until assayed. Pituitary hormone concentrations in serum were measured by radioimmunoassays using ^{125}iodine-labelled tracers. LH–RH concentrations were determined by a radioimmunoassay without extraction in plasma samples treated with 0·05 M benzamidine as peptidase inhibitor. The radioimmunoassay was developed by Dr. K. Reber of our department with antibodies generated in rabbits against unconjugated LH–RH. The tracer was chloramine T-iodinated (^{125}iodine) LH–RH used after two separations on a Sephadex G 25 column. Testosterone/5α-dihydrotestosterone concentrations were measured by radioimmunoassay with antibodies raised in rabbits against testosterone-7α-carboxymethylthioether coupled to bovine serum albumin. The tracer was [1,2,6,7(n)-^{3}H]testosterone (Radiochemical Centre, Amersham, England).

The results of the first experiment are summarized in Table 1. The animals of the control and first experimental groups bore a subcutaneous implant of LH–RH over a period of eight days. The implants of the third group were removed after 8 days and these animals decapitated eight days later. The fertility of each animal was tested one day before decapitation by placing a pro-oestrous female in its cage. Serum LH concentrations in the LH–RH group were doubled after eight days, whereas the FSH concentrations were halved. Testosterone/5α-dihydrotestosterone concentrations as well as testicular weight and fertility were markedly reduced. Eight days after removal of the implants the absolute FSH levels were higher than those in the group bearing empty implants, although a strict comparison is precluded by the dissimilarity in observation times. Testicular weights were low and fertility was still reduced.

In a second experiment (Table 2) we studied, in addition to LH–RH, the effectiveness of a TRH implant and the possibility of re-using the implants. LH–RH had the same effect on testicular weight as in experiment I, while TRH led to a statistically significant hypertrophy of the thyroid gland. The effects of LH–RH on circulating hormone concentrations are the same as those in experiment I; the TSH concentrations in the TRH group were increased while serum prolactin concentrations were unchanged.

Our results show that two oligopeptides can be released from silicone rubber over a period of at least ten days. The material incorporated showed no signs

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† Synthesized by Drs. R. Studer and D. Gillessen (Diagnostic Research Department).

Table 1. Organ weights, serum hormone concentrations and fertility of male rats (n = 8) bearing subcutaneous implants with or without 10% LH–RH over a period of eight days and eight days after removal of the implants

		Organ weights			Serum hormone concentrations			
Treatment with s.c. implants	Testes (g)	Seminal vesicles (mg)	Ventral prostate (mg)	Musculus levator ani (mg)	LH (ng ml^{-1})	FSH (ng ml^{-1})	Testost. + DHT (ng ml^{-1})	Fertility (pregnant females/ total)
Empty (8 days)	2·5 ± 0·1	159 ± 10	199 ± 15	145 ± 8	48 ± 5	384 ± 32	4·4 ± 0·6	8/8
10% LH–RH (8 days)	1·8 ± 0·2***	129 ± 7*	166 ± 11 ns	159 ± 14 ns	91 ± 14***	158 ±30***	1·1 ± 0·1***	5/8
10% LH–RH (8 days) followed by 8 implant-free days	1·9 ± 0·2	172 ± 11	209 ± 13	137 ± 8	39 ± 7	505 ± 45	3·9 ± 0·6	5/8

Results are mean values ± s.e.m. (ns = statistically not significant, *$P<0·05$, ***$P<0·001$).

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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REFERENCES

Auclair, C., Kelly, P. A., Coy, D. H., Schally, A. V., Labrie, F. (1977) Endocrinology 101: 1890–1893

Corbin, A., Beattie, C. W. (1975) Endocrinol. Res. Commun. 2: 445–448

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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