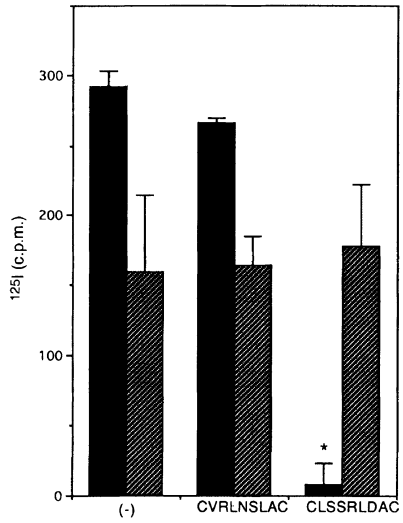

FIG. 4 Tissue localization of red blood cells coated with CLSSRLDAC peptide. Inhibition of brain localization by the corresponding soluble peptide. Iodinated CLSSRLDAC peptide was coupled to red blood cells and injected into the tail vein of mice in the presence or absence of unlabelled peptides. Radioactivity in perfused brain (black bars) and kidney (hatched bars) tissues is shown.

METHODS. The CLSSRLDAC peptide (1 mg) was labelled using the Bolton Hunter reagent (Amersham Life Science, Arlington Heights, IL), and purified by reversed-phase chromatography on Sep-Pak cartridges (Waters, Millipore, Milford, MA). The labelled peptide (100 μg) was coupled to 1 ml glutaraldehyde-stabilized sheep red blood cells (Sigma, St Louis, MO) according to the manufacturer's instructions. The coated cells (50 μl, 200,000 c.p.m.) were injected into the tail vein of mice in the presence or absence of 10 mM of unlabelled CLSSRLDAC peptide. An unrelated peptide, CVRLNSLAC, was used as a control. The mice were killed 2 min later, perfused through the heart with 50 ml DMEM, and their brain and kidneys were removed and assayed for radioactivity. The animals were treated in accordance with the Institute's Animal Facility Guidelines. Bars show s.e.m. from triplicates. Asterisks indicate statistically significant differences (unpaired Student's *t*-test, $P < 0.05$).

sequence and of the WRCVLREGPAGGCAWFNRHRL phage, but had no effect on the brain localization of the CENWWDGVC phage (Fig. 3). Thus the first two peptides, which were obtained from different libraries in two independent experiments, seem to bind to the same target molecule, possibly because of the similarity of the C terminus of the long peptide with the SRL motif. Differences in binding parameters may explain the greater susceptibility of the long motif to inhibition by the CLSSRLDAC peptide. The third peptide is likely to have a different target.

We also showed that CLSSRLDAC could target a particle other than the phage to the brain. Coupling the peptide onto the surface of red blood cells resulted in their accumulation in the brain to a greater extent than in the kidney (Fig. 4). Moreover, the brain localization of the red blood cells was blocked by co-injection of the soluble peptide, whereas the accumulation in the kidney was not affected (Fig. 4).

Future studies will be needed to identify the molecules to which the peptides bind in the brain and kidney. The sequences of the binding motifs are not helpful in this regard, because they do not reveal any significant similarities with known receptor ligands. Our initial attempts to identify the target molecule for the CLSSRLDAC peptide by affinity chromatography of brain extracts have not been successful, possibly because endothelial cell molecules would only be present as minor components in a brain extract. The receptors for the peptides are likely to be endothelial cell molecules, because the phage were allowed to circulate only for a few minutes, making it unlikely that the phage would have left the circulation. Moreover, immunohistochemical staining of phage after injection showed that they remain in the lumen of blood vessels in the targeted organs.

To our knowledge this is the first time an *in vivo* selection procedure has been applied to a random library. So far we have targeted only two organs, the brain and the kidney, and were in each case able to recover organ-selective phage. This initial success suggests that it will be possible to apply this procedure to the identification of selective binding sequences for other organs as well, although organs that capture a large number of phage, such as liver and lung, may prove rather more troublesome. The method should be applicable to phage display libraries expressing larger proteins including the antibody variable binding region and the binding domains of specific ligands, as well as random libraries based on principles other than phage display; the only requirement is the ability to identify the compound in the tissue after the *in vivo* binding.

Organ-selective targeting molecules isolated from random libraries following the procedures described here may have a variety of uses. It may be possible to graft motifs to surface molecules of viruses or cells used in gene therapy. Other possibilities include their use in the preparation of drug conjugates or liposomes with specific targeting properties. Tumour vasculature, which undergoes active angiogenesis and contains specific markers[14,15], would be a particularly attractive future target, as it might allow therapies to be directed into tumours while sparing other tissues. □

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CORRECTION

Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice

Lutz Hein, Gregory S. Barsh, Richard E. Pratt, Victor J. Dzau & Brian K. Kobilka

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22. Nakajima, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **92,** 10663–10667 (1995).
25. Viswanathan, M. & Saavedra, J. M. *Peptides* **13,** 783–786 (1992). □