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A Rapid Method, Using Octadecasilyl-Silica, for the Extraction of Certain Peptides from Tissues

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Peptides can be recovered from tissues by homogenization in a carefully selected extraction medium followed by adsorption from the supernatant on a small bed of octadecasilyl-silica. Recoveries of corticotropins and somatostatin added to a variety of tissues were quantitative, and the peptides undamaged as determined by high-pressure liquid chromatography.

Octadecasilyl-silica is a useful solid phase for high-pressure liquid chromatography (h.p.l.c.) of peptide hormones (Gruber *et al.*, 1976; Burgus & Rivier, 1976; Bennett *et al.*, 1977) and it can also be used for efficient and rapid extraction of peptides and peptide fragments from plasma (Bennett *et al.*, 1977). Whole plasma or plasma that has been deproteinized with trifluoroacetic acid is passed through a 0.5–2ml bed of octadecasilyl-silica in a syringe, the bed is washed and adsorbed peptides eluted with a mixture of methanol, water and trifluoroacetic acid. This procedure does not cause damage to peptides, gives good yields and in addition it is convenient for preparing samples for octadecasilyl-silica chromatography, since the eluate after dilution with 1% trifluoroacetic acid can be pumped directly on the chromatographic column (Bennett *et al.*, 1977).

To apply this type of extraction procedure to tissue samples it was necessary to find a homogenization medium that would satisfy a number of conditions. It should provide an aqueous supernatant containing most of the tissue peptide. The supernatant must not occlude the extraction column and peptides in the supernatant must readily adsorb on the octadecasilyl-silica. In addition, the medium must suppress the degradation of peptides by enzymes released during homogenization. The present paper describes the development of a medium for tissue homogenization that satisfies these conditions and in conjunction with the use of octadecasilyl-silica has proved suitable for the extraction of corticotropins and somatostatin from a variety of tissues.

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Materials and Methods

Materials

Disposable 5ml syringes for the extraction column were obtained from Gillette Surgical, Isleworth, Middx., U.K. Porous Teflon discs were cut from sheets obtained from Aeros Plastics, Stroud, Glos., U.K. Pre-packed octadecasilyl-Partisil (particle size 10μm) columns were obtained from Reeve Angel, London S.E.1, U.K. Porasil A (35–70μm mesh) was from Waters Associates, Northwich, Cheshire, U.K. and trichlorooctadecylsilane was from Aldrich Chemical Co., Gillingham, Dorset, U.K. Trifluoroacetic acid was obtained from Fisons, Loughborough, Leics., U.K. Human corticotropin and corticotropin analogues were prepared labelled with tritium in specific positions by catalytic replacement of iodine (Brundish & Wade, 1973, 1976; Brundish *et al.*, 1976; Brundish & Wade, 1977). [[4-^{3}H]Phe^6]Somatostatin was prepared by the method of Allen, *et al.* (1978).

Where possible, other reagents were analytical-reagent grade.

Methods

Male Wistar rats (300g) were killed by decapitation and samples of kidney, liver, small intestine and muscle were removed and placed in homogenization medium at room temperature (10ml of medium for each gram of tissue). As a result of experiments with a number of media the solution selected as a homogenization medium consisted of 50ml of formic acid, 150ml of trifluoroacetic acid, 10g of NaCl and 91ml of 11M-HCl made up to 1 litre with water. Labelled peptide was added and the tissue finely chopped with scissors as required, homo-

genized in a type PT120 Polytron homogenizer (The Northern Media Supply, Hull, U.K.) at setting 5 for 1min, and centrifuged at 4000*g* for 10min. In experiments where recoveries were important the pellet was re-extracted by homogenization with an equal volume of extraction medium and then centrifuged under the same conditions. The supernatant(s) (20–40ml) was strained through a nylon tea-strainer, then through 20μm nylon gauze and 5ml portions were loaded on the extraction column (a 1ml bed of octadecasilyl-Porasil). Octadecasilyl-Porasil was prepared as described by Bennett *et al.* (1977). The extraction bed was packed in a 5ml plastic syringe between two porous Teflon discs. The sample was passed through the column twice, then the column was washed with 5ml of 1% trifluoroacetic acid and eluted slowly with 5ml of methanol/water/trifluoroacetic acid (80:19:1, by vol.). The eluate was diluted with 15ml of 1% trifluoroacetic acid and could be pumped directly on an octadecasilyl-silica h.p.l.c. column for peptide separation. The extraction column was washed with 10ml of the elution medium and 10ml of 1% trifluoroacetic acid, ready for extraction of a fresh sample. Extraction columns could be used for several samples but eventually became occluded.

Results and Discussion

Experiments with a number of simple aqueous solutions as homogenization media showed that 5% formic acid had useful properties. It suppressed degradation of corticotropin-(1–24)-tetracosapeptide in muscle and liver homogenates and minimized losses of peptide in the precipitate. However, this medium was found to be inadequate in a number of ways and was therefore modified. Extraction from the supernatant onto octadecasilyl-silica was inefficient, but could be improved by addition of 1% NaCl. It is suspected that the peptides may have been bound to proteins by ionic bonding. When a larger number of tissues was tested it was found that human corticotropin was degraded in kidney homogenates and this could be prevented by incorporation of HCl to a final concentration of 1M. Finally, a tendency for supernatants to block the extraction column was overcome by deproteinization with 15% trifluoroacetic acid. We have found trifluoroacetic acid to be more useful than trichloroacetic acid for deproteinizing peptide solutions since peptide tends to precipitate with the protein when the latter reagent is used.

As a result of these observations the medium used for the extraction experiments described below consisted of aq. 5% formic acid (v/v), 15% trifluoroacetic acid (v/v) and 1% NaCl (w/v) containing 1mol of HCl/litre. Chromatograms showing the integrity of corticotropin-(1–24)-tetracosapeptide after extraction are shown in Fig. 1. The chromatographic system separates products such as the sulphoxide and the 3–24 and 1–20 peptide fragments of the parent molecule (Hudson *et al.*, 1977). Recoveries of different corticotropins and somatostatin from a number of tissues are shown in Table 1. For the corticotropins the extraction procedure involved a further step in which the effluent from the extraction column was further extracted on a second column to yield a further few per cent of peptide. The somatostatin was apparently unchanged as judged by h.p.l.c. on octadecasilyl-silica as described by McMartin & Purdon (1978). Although the results

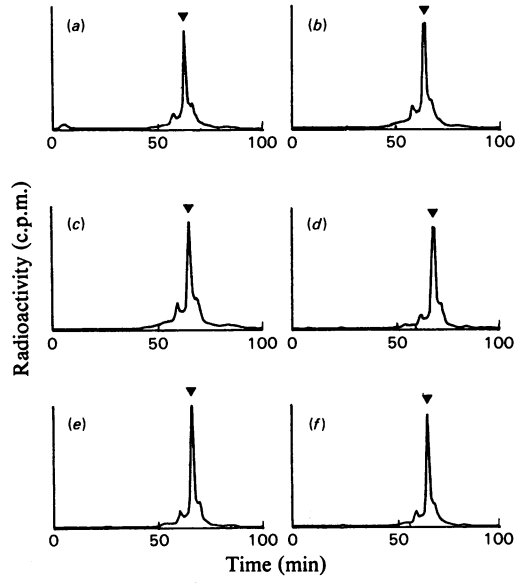

Fig. 1. *H.p.l.c. of* [^3H]*corticotropin-(1–24)-tetracosapeptide after recovery from different tissues of the rat*
A mixture containing corticotropin-(1–24)-tetracosapeptide labelled in tyrosine-2, phenylalanine-7 and tyrosine-23 (total peptide 25μg) was added to 2g of either kidney (*b*), liver (*c*), intestine (*d*), skin (*e*) or muscle (*f*), and the tissue extracted as described under 'Methods'. The extracts were applied to a high-resolution octadecasilyl-Partisil (10μm) column (4mm×250mm), which was eluted with a linear gradient (combined volume 100ml) from methanol/water/trifluoroacetic acid (20:79:1, by vol.) to methanol/water/trifluoroacetic acid (55:44:1, by vol.) at a flow rate of 0.7ml/min. Radioactivity was determined by liquid-scintillation counting of 0.5ml fractions. (*a*) is a control chromatogram of labelled peptide that had not been added to a tissue and extracted. The filled triangle above each chromatogram indicates the position of elution of corticotropin-(1–24)-tetracosapeptide.

Table 1. *Recoveries of various labelled peptides added to tissues after extraction with octadecasilyl-silica*

The extraction medium contained 5% formic acid (v/v), 15% trifluoroacetic acid (v/v), 1% NaCl (w/v) and 1 M-HCl for homogenization followed by adsorption and elution on octadecasilyl-silica. The quantities of peptide added were in the range of 1 to 10 μg/g of tissue.

Peptide	Tissue	Recovery (%)
[[3,5-$^3H_2$]Tyr^2]Corticotropin-(1–24)-tetracosapeptide	Kidney	84
	Small intestine	76
	Liver	83
[[3,5-$^3H_2$]Tyr^{23}]Corticotropin (human)	Kidney	90, 93, 94
[D-Ser^1, [4,5-$^3H_2$]Tyr^2, Lys^{17}, Lys^{18}]Corticotropin-(1–18)-octadecapeptide amide	Kidney	89
	Kidney	89
[[4-^{3}H]Phe^6]Somatostatin	Kidney	90, 88
	Muscle	88, 84
	Lung	94, 83
	Liver	84, 85

shown in Fig. 1 and Table 1 are obtained with μg amounts of peptides, 1 g of octadecasilyl-silica will extract quantities up to 2–3 mg.

This extraction procedure is based on general principles that should apply to a variety of peptide hormones. The suppression of proteolytic activity during homogenization is adequate for corticotropin, which is one of the most labile hormones. Most peptide hormones possess hydrophobic amino acid residues that are required for adsorption on octadecasilyl-silica. Difficulties may be experienced with larger hormones that are likely to precipitate along with proteins during homogenization. For peptides where 15% trifluoroacetic acid (v/v) causes losses due to precipitation it may be possible to improve recoveries by increasing the extraction volume, by lowering the trifluoroacetic acid concentration or even by omitting this reagent altogether.

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