The effect of dietary fibre on ^{85}Sr and ^{47}Ca absorption in infant rats[1]

B. Momčilović[2] and Nevenka Gruden

Institute for Medical Research and Occupational Health, M. Pijade 158, 41000 Zagreb (Yugoslavia), 6 September 1980

Summary. The retention of bone-seeking radioisotopes ^{85}Sr and ^{47}Ca was about 20% lower in the femur of 6-day-old infant rats artificially fed on milk, milk supplemented with calcium and phosphorus, and milk supplemented with strontium when 6% cellulose type dietary fibre was added to the diet.

The high radiotoxicity of strontium in the very young is well documented[3,4]. Simultaneous administration of calcium, phosphorus, and alginates was found to be the best method for decreasing radiostrontium absorption from the gastrointestinal tract of infant rats[5,6]. Recently it has been demonstrated that dietary fibre could reduce the absorption of calcium, zinc, and some other elements from the diet[7–9]. The aim of this experiment was therefore to investigate the effect of dietary fibre on the absorption of radioactive strontium and calcium from the gastrointestinal tract of infant rats.

Methods and results. The experiment was performed on 54 6-day-old infant rats in litters of six. Each littermate was randomly assigned[10,11] to 1 of the 6 available treatments: A) Milk (pasteurized cow's milk containing 140 mg Ca and 95 mg P/100 ml as analyzed by standard methods)[12,13]; B) milk + 6% cellulose type dietary fibre (Tekland Mills, Division of ARS/Sprague Dowley, Madison, Wisconsin, USA); C) milk + Ca + P (260 mg Ca from $CaCl_2 \cdot 2\,H_2O$ and 135 mg of P from $KHPO_4$ were added to 100 ml of pasteurized cow's milk so that the final content of 400 mg Ca and 235 mg P per 100 ml adequately simulated rat's milk)[14]; D) milk + Ca + P + fibre; E) milk + Sr (57 mg from $SrCl_2 \cdot 6\,H_2O$/100 ml of pasteurized cow's milk, i.e., a dose comparable with the experiment of Harrison and co-workers)[15]; and F) milk + Sr + fibre. Standard and semipurified experimental diets contained 6% and 3% of dietary fibre respectively[7,16]. The animals were artificially fed 0.45 ml of the respective diet marked with 1 µCi ^{85}Sr and 1 µCi ^{47}Ca/ml with a dropper for a period of 6 h[17]. The isotopes were in an almost carrier-free form (Radiochemical Centre, Amersham, England). The radioactivity of ^{85}Sr and ^{47}Ca in the whole body and femur were determined 6 days later in a single-channel twin crystal or a well-type scintillation counter NaI (T1) respectively (Nuclear Chicago, USA)[18,19]. The results are expressed in percentage of the administered dose as an arithmetical mean with a standard error. The data were classified simultaneously according to the effect of fibre and the effect of mineral supplementation[20], whereas the significance of the difference among the groups was determined by Duncan's multiple range and multiple F tests[21].

In general, regardless of mineral supplementation, dietary fibre decreased ^{85}Sr and ^{47}Ca retention in both the whole body ($p < 0.05$) and femur ($p < 0.01$) of infant rats (table). Interpair comparisons revealed that the retention of both bone-seekers was 20% lower in the femur of infant rats fed the diet containing fibre ($p < 0.05$ for diets A vs B, C vs D, and E vs F respectively) but only slightly affected in the whole body ($p < 0.10$ for respective pairs). The addition of calcium and phosphorus (diet C) decreased ^{85}Sr and ^{47}Ca in the examined tissues and the lowest retention of both radionuclides was observed when infant rats were fed milk containing additional calcium, phosphorus, and fibre (diet D). The amount of stable strontium as used in this experiment (diet E) appears of marginal significance. The $^{85}Sr/^{47}Ca$ ratio was unaffected by the type of the dietary treatment (pooled value $X_{54} = 0.89 \pm 0.01$).

The effect of dietary fibre, calcium, phosphorus, and strontium on ^{85}Sr and ^{47}Ca absorption in infant rats

	% Dose* $^{85}Sr^a$ Milk**	Milk + Ca + P***	Milk + Sr****	$^{47}Ca^b$ Milk	Milk + Ca + P	Milk + Sr
Whole body						
Control[c]	64.22 ± 0.78^{g}	$60.99 \pm 1.02^{h,i}$	64.01 ± 1.07^{g}	73.10 ± 0.87^{g}	$69.78 \pm 1.43^{h,i}$	$72.78 \pm 1.25^{g,h}$
*****Fibre[d]	$61.67 \pm 1.18^{g,h,i}$	59.32 ± 1.08^{i}	$62.56 \pm 0.64^{g,h}$	$72.75 \pm 0.94^{g,h}$	68.62 ± 1.50^{i}	$71.24 \pm 0.63^{g,h,i}$
Femur						
Control[e]	1.17 ± 0.04^{g}	0.82 ± 0.05^{i}	1.02 ± 0.07^{h}	1.30 ± 0.07^{g}	0.90 ± 0.06^{j}	$1.17 \pm 0.07^{g,h}$
Fibre[f]	$0.96 \pm 0.05^{h,i}$	0.61 ± 0.05^{j}	0.84 ± 0.03^{i}	$1.08 \pm 0.07^{h,i}$	0.66 ± 0.05^{k}	$0.94 \pm 0.04^{i,j}$

* Mean ($\bar{X}_9$) ± SE. ** Containing 95 mg P and 140 mg Ca/100 ml, *** Containing 235 mg P and 400 mg Ca/100 ml, **** Containing 57 mg Sr/100 ml, ***** Containing 6000 mg cellulose type dietary fibre/100 ml. [a,b] $p < 0.05$; [c,d] $p < 0.05$; [e,f] $p < 0.01$; [g–k] means bearing various superscripts for the same tissue and isotope differ significantly ($p < 0.05$).

Discussion. Kostial and co-workers[22] demonstrated that the addition of calcium and phsphorus to milk decreased ^{85}Sr and ^{47}Ca absorption from the gastrointestinal tract of infant rats and that the greatest reduction of ^{85}Sr and ^{47}Ca retention was observed when calcium, phosphorus, and 20% alginates were administered simultaneously[5,6]. However, O.G.1, an alginate containing 97% of guluronic acid[23] selectively inhibited strontium absorption[5,6] whereas the treatment with the low level cellulose type dietary fibre interfered with both strontium and calcium absorption. Considering the fact that the femur, as compared to the whole body, is a tissue of choice for assessing skeletal deposition of bone-seeking isotopes[24] from the diet[25,26], it is reasonable to assume that the deposition of ^{85}Sr and ^{47}Ca in the femur reflects actual differences in their absorption from the gut. The mechanism by which cellulose decreases ^{85}Sr and ^{47}Ca absorption from the gastrointestinal tract of infant rats remains to be elucidated. Dietary fibres are a heterogenous group of plant constituents including cellulose, hemicellulose, lignin, pectin, gum, and mucilage[27], and purified cellulose might hold water through entrapment but probably does not show a great adsorptive or ionexchange capacity[28]. However, cellulose is the fibre most similar to alginates, the properties of which appear to differ from those of cellulose mainly because of the carboxyl groups, whereas the hydroxyl group determines moisture, absorbancy and other cellulose qualities[29].

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Effect of substitution of glycine by D- or L-alanine on the activity of the C-terminal hexapeptide analogue of substance P on isolated guinea-pig ileum

A.W. Lipkowski, Stefania Drabarek, T. Majewski, Anna Maria Konecka and B. Sadowski

Peptide Laboratory, Institute of Fundamental Problems of Chemistry, Warsaw University, ul. Pasteura 1, PL-02-383 Warsaw (Poland), and Department of Behavioral Physiology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, PL-05-551 Jastrzębiec (Poland), 6 June 1980

Summary. Two new C-terminal hexapeptide analogues of substance P having D- or L-alanine in position 9 were synthetized. Their contracting activities on isolated guinea-pig ileum were considerably lower than that of < Glu-SP^{7-11}.

Substance P (SP), a naturally occurring undecapeptide was discovered in the brain and equine gut by von Euler and Gaddum[1] in 1931. However it was not purified until 1970 when Chang et al.[2] isolated substance P from the hypothalamus and determined the amino acid sequence of this peptide[3] (table 1, **I**).

The C-terminal hexapeptide fragment of substance P – SP^{6-11} (**II**) and its pyroglutamyl-analogue (< Glu-SP^{7-11}, **III** in table 1) show the full activity of the native undecapeptide in most biological tests[4,5]. The same holds true for nonmammalian tachykinins such as eledoisin or physalaemin[6]. All these tachykinins contain a glycine residue in position 9 which may account for their biological activity[7].

The purpose of this study was to find out to what extent a substitution of glycine in position 9 by other amino acids could affect the biological activity of the C-terminal hexapeptide analogue of SP on the isolated guinea-pig ileum.

Material and methods. 1. Peptides. We synthetized 2 new peptides, **IV** and **V**, whose chemical structure is presented in table 1, by a 3+3 fragment coupling method (fig. 1).

The method involved the same protecting groups, coupling procedures and purification as was described for **III**[8]. The physico-chemical properties of the peptides **IV** and **V** are summarized in table 2.

2. Bioassay. The investigations were performed on guinea-pig ileum according to Yau[9]. 3-month-old guinea-pigs of either sex were anaesthetized with chloroform. A 3-3.5-cm-long segment of ileum was removed, washed and suspensed in 50 ml of Krebs solution at 37 °C. A 95% O_2-5% CO_2 mixture was bubbled through the bath. The peptides were dissolved in dextran-water solution and diluted with Krebs

Table 1. Amino acid sequences of substance P, C-terminal hexapeptide fragment and its analogues

Number	Peptide
I	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
II	Gln-Phe-Phe-Gly-Leu-Met-NH_2
III	< Glu-Phe-Phe-Gly-Leu-Met-NH_2
IV	< Glu-Phe-Phe-D-Ala-Leu-Met-NH_2
V	< Glu-Phe-Phe-L-Ala-Leu-Met-NH_2

Abbreviation: < Glu = pyroglutamyl.