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SHORT COMMUNICATIONS

Occurrence of bradykinin in human pulmonary carcinoma

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THE PRESENCE of polypeptides with kininic activity was previously demonstrated in this laboratory in some pathological conditions in animals and man.[1, 2]

Tissues capable of rapid and vigorous protein synthesis and rich in proteolytic enzymes, such as animal and human neoplastic tissues with very rapid cell multiplication, could be potential sources of biologically active peptides. In view of this possibility, we carried out investigations on a series of human adenocarcinomas from lung, stomach, kidney, large intestine, made available through surgical intervention.

The tissues immediately after removal were frozen in liquid nitrogen and then preserved until the gathering of a sufficient quantity of material for the extraction of the polypeptides.

The biological material was slowly brought to room temperature in the course of about 2 hr by means of controlled raising of the temperature of the freezing room and it was then homogenized in glacial acetic acid at a constant temperature of 15°.

The acetic extract was diluted at a concentration of about 1 M in acetic acid and then dialysed in Visking tubes against a 1 M solution of acetic acid for 48 hr with 12 changes of the liquid of dialysis.

The dialysed material was lyophilized at 0°. It was then recovered in absolute ethanol and transferred on a column of basic aluminum 80 cm in height and 2·5 cm in diameter which had previously been activated and washed with absolute ethanol. After the extract had settled, the column was again washed with absolute ethanol (200 ml) and then chromatography was started with gradients of ethanol in water from 95 to 30%. The fractions, up to the concentration of 70%, were discarded because they contained some biogenic amines. When the main biological activity of presumed peptides was assayed on the intestine of guinea pig ileum, it was found to be mainly distributed in a broad band in the fraction from 60% to lower concentrations, with maximal activity in the fraction of ethanol at 40%. The fractions with biological activity were then evaporated at low temperature and then submitted again to chromatography on a column of DEAE Sephadex and eluted with pyridine acetate 0·1 M at pH 4·5.

The fractions which contained the biological activity and gave a positive reaction to ninhydrin before and after hydrolysis, were then collected and pooled. Subsequently, these fractions were fractionated by means of counter-current distribution, using the system *n*-butanol-ethanol-acetic acid-water with a total of 200 "transfers". In this way we obtained five ninydrin positive fractions A_1, A_2, A_3, A_4, A_5, which were then tested for their biological activity on isolated organs and on laboratory animals.

In the process of fractionation of lung tumours, the biological activity as tested on isolated organs was found almost entirely in the fraction A_1, which was then submitted to further purification. The fraction was submitted to electrophoresis on starch gel and then again analysed with electrophoresis on cellulose acetate. The fraction seemed to move as a unique band almost identical to that of bradykinin.

No difference was noted as compared to the standard of bradykinin, when the terminal pipsyl-peptides of the two peptides were synthesized with the reaction of the p-I^{131}-phenyl sulphonic-acid chloride and then submitted to chromatography on a thin layer of silica gel with a mixture of ether–ethanol–acetic acid, with the technique previously described.[3]

This procedure, when applied to about 3·500 g of lung tumour, led to the isolation of about 9 g of a peptidic fraction A_1, in which was found the maximal biological activity of the extract, on

isolated organs, while the pain-producing activity was scarce in this fraction and much higher in the total extract. The biological date of the titration of this fraction, as well as of the titration of the total extract of the other fraction, are reported in Table 1.

TABLE 1. BIOLOGICAL EFFECTS OF POLYPEPTIDE FRACTIONS FROM HUMAN PULMONARY CARCINOMA

Counter-current fractions	Dog pressure (μg. brad/ml)	Rabbit pressure (μg. brad/ml)	Rat uterus (μg. brad/ml)	Guinea pig ileum (μg. brad/ml)	Rabbit large bowel (μg.fsl/ml)	Rat large bowel (μg. eled/ml)	Rat writing reflex (for pain measure) (μg. brad/ml)
Total extracts	+++	++	+++	+	—	—	+++
A_1	20	20–25	25	20	<0.05	<0·2	+
A_2	1–2	0·2	3	0·3	<0·05	<0·2	—
A_3	0	0·4	0·15	0·3	<0·05	<0·2	—
A_4	0	0·2	0·1	0·3	<0·05	<0·2	—
A_5	0	0·2	0·15	0·3	0·1	<0·2	—

Brad—bradykinin; fisl—physalemin; eled—eledoysin.

The fraction A_1 was submitted to aminoacid analysis, according to Moore and Stein procedure. The results of aminoacid analysis confirm the biological tests and the electrophoresis analysis performed on the peptide, giving an aminoacid composition almost identical to that of pure bradykinin: a polypeptide of nine aminoacids: 2 arginine, 1 serine, 3 proline, 1 glycine, 2 phenylalanine.

TABLE 2

Amino acid	Base line	Height	Half height	H net height	Micro moles H × W C		
Arginine	0	12806	—	12806	2·28	1·70	2
Serine	+ 1	6778	—21	6757	1·36	1·02	1
Proline	+ 1	4148	—35	4113	3·66	2·73	3
Glycine	+ 2	6267	—54	6213	1·32	0·98	1
Phenylalanine	+10	12574	—390	12184	2·55	1·90	2

Results—Arg_2, Ser_1, Pro_3, Gly_1, Phe_2

The analysis has been confirmed by the *Instituto Regina Elena per lo Studio del cancro* and by the *Institut für organische chemie der Universität Basel.*

Bradikininogen in normal lung and in tumour was titrated with the method of Rocha and Silva. The results are listed in Table 3.

TABLE 3. BRADIKININOGEN CONTENT OF HUMAN LUNG CARCINOMA

	Bradikininogen content
Human plasma	13 mcg/ml
Normal lung	0·8 mcg/g
Lung carcinoma	4·5 mcg/g

Values in mcg of released bradikinin upon digestion with trypsin.

It appears that bradikininogen content in lung adenocarcinoma is far more copious than that found in normal lung although lower than in normal plasma.

From the biological point of view a question arises on the nature of bradykinin in lung since this peptide was found to occur in human blood in minimal amount, a fact that led to the hypothesis that the biological activity recovered in lung extracts could be due to the bradykinin content of the blood always present in the tissue. Indeed the peroxydase test performed on our extracts gives a content of blood in lung of about 800 ml/3·500 g of tissue.

Since biological tests on ethanol extracts of total blood give a figure of about 10 ng of bradykinin per 100 ml of blood, the maximal amount of blood bradykinin in our extracts could be of 8 μg per 3·500 g of tissue.

This finding corresponds to about one thousandth of the content of bradykinin recovered by us in lung carcinoma and makes rather unlikely the hypothesis of a haematic origin of lung bradykinin.

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Uncoupling of oxidative phosphorylation by some fluoro-compounds, notably perfluoropinacol

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RECENT reports of the potent herbicidal and uncoupling activity of trifluorobenzimidazoles[1, 2] indicate the importance of the strongly electrophilic trifluoromethyl group, which confers sufficient acidic character to render these compounds potent drugs. Many other non-phenolic acidic compounds possess this ability to uncouple oxidative phosphorylation, e.g. thiols,[3] β-diones,[4] triazoles,[5] carbonyl-cyanide phenylhydrazones.[5, 6] This report indicates that an acidic (and toxic) aliphatic secondary alcohol, dodecafluoropinacol (pK, 6·0),[7] is also a very potent uncoupling agent.

MATERIALS

The perfluoropinacol–dioxane complex (m.p. 80°) and hexafluoroacecetone sesquihydrate were kindly supplied by Dr. W. J. Middleton (Central Research Dept., E. I. du Pont de Nemours & Co., Wilmington, Delaware, U.S.A.); pentafluorophenol and pentafluorobenzoic acid by Dr. A. E. Pedler (Chemistry Dept., Birmingham University); other fluorophenols and fluorothiophenols by Mr. D. S. Robinson and Dr. A. K. Barbour (Imperial Smelting Corp. Ltd., Bristol 1); hexafluoro-2-phenylisopropanol by Dr. A. E. Tyczkowski (Hynes Chemical Research, Durham, North Carolina, U.S.A.); N-(α,α,α-trifluoro-*m*.tolyl)-anthranilic acid (Flufenamic acid) by Dr. J. L. Gorringe (Parke, Davis & Co. Ltd., Hounslow). *m*.α,α,α-Trifluoromethylphenol was prepared from $\alpha.\alpha.\alpha$-trifluoro-*m*.toluidine.[8] Other fluoro-compounds were obtained from Kodak Ltd., Liverpool; British Drug Houses, Poole and Koch-Light Ltd., Colnbrook, Bucks.