on the response of bone alkaline phosphatase to parathyrin. The results of a typical experiment showing percentage change in alkaline phosphatase activity compared with the hormone-free control are presented in Fig. 2(*b*). Tissue from 1-day-old animals was unresponsive to parathyrin, that from the 3-day-old animal showed decreased alkaline phosphatase activity and those from 5- and 6-day-old animals showed an increase in alkaline phosphatase activity. All responses were maximal at 5–8 min incubation time. In bone from 5-day-old rats a dose-dependent increase in enzyme activity was observed at 7 min in the range 50 fg/ml–50 pg/ml (Fig. 2*a*).

This study highlights the potential of quantitative cytochemistry for studying enzyme characteristics intracellularly without prior fractionation and purification. Because of the micro-quantities of tissue involved, it becomes possible to measure changes in cellular enzyme activity caused by sub-picogram quantities of hormone. The present results of the effect of parathyrin on bone alkaline phosphatase introduce the prospect of a bioassay of greater sensitivity than the existing radioimmunoassay methods.

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Immunological Characterization of Two Substance-P Antisera with Substance-P Fragments and Analogues

DERMOT CANNON, PETR SKRABANEK and DAVID POWELL

Endocrine Unit, Mater Hospital, Dublin 7, Ireland

and M. G. HARRINGTON

Department of Biochemistry, University College, Belfield, Dublin 4, Ireland

Substance P is an undecapeptide, L-Arg-L-Pro-L-Lys-L-Pro-L-Gln-L-Gln-L-Phe-L-Phe-Gly-L-Leu-L-Met, which has been isolated in pure form, sequenced and synthesized. The availability of the peptide in pure synthetic form has permitted immunization of guinea pigs for antibody production. The present paper shows the results of a study on the structural basis of antibody–antigen interactions in our radioimmunoassay system for substance P by using two different high-affinity antisera and various tracer analogues, multiple-synthetic fragments and related peptides.

Substance P conjugated to bovine immunoglobulin was used for immunization of guinea pigs. An analogue of substance P with tyrosine substituted for phenylalanine at residue 8 ([Tyr^8]-substance P) was labelled with ^{125}I. Substance P with tyrosine attached to the *N*-terminus (Tyr-substance P) and the related physalemin peptide were also labelled. Concentration of substance P was determined by radioimmunoassay with a sensitivity of 2 pg (O'Connell *et al.*, 1976). Appropriate control tubes containing all constituents except antiserum were included to determine damage of tracer (i.e. apparent binding of tracer in the absence of antibody). Both antisera were used at a final dilution of 1 : 500000. Substance-P fragments and the related peptides physalemin and eledoisin were prepared in assay buffer (0.05 M-sodium barbital, pH 8.6, containing 2.5% pooled plasma). Assay incubation time was 20 h at 4°C.

The substance-P-(4–11)-octapeptide fragment gave dose–response curves similar to the synthetic standard, whereas the substance-P-(6–11)-hexapeptide fragment was more potent in its ability to displace tracer (Fig. 1). Physalemin displaced the [Tyr^8]-

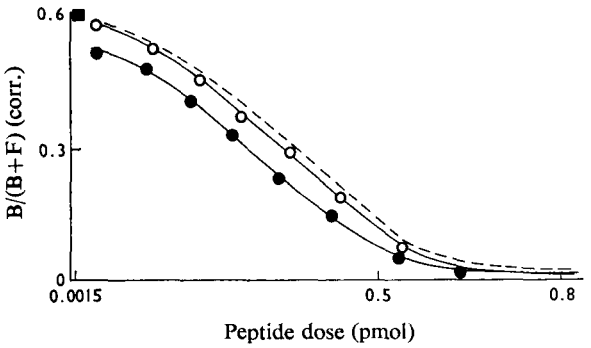

Fig. 1. *Dose–response curves for substance P* (○), *substance-P-*(4–11)*-octapeptide* (----) *and substance-P-*(6–11)*-hexapeptide* (●)

■, Antibody control. B/(B+F) is the ratio antibody-bound radioactivity:total radioactivity. The values of the ratio shown are for a typical experiment.

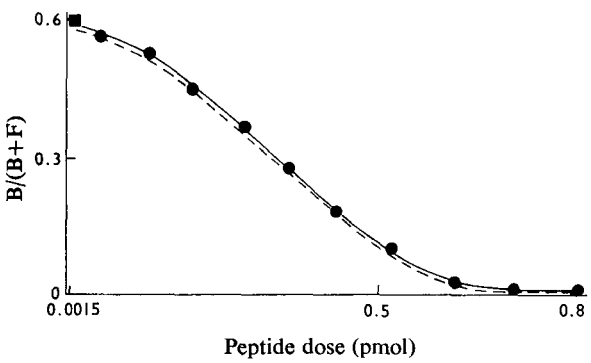

Fig. 2. *Dose–response curves for substance P* (●) *and physalemin* (----)

■, Antibody control. B/(B+F) is the ratio antibody-bound radioactivity:total radioactivity. The values of the ratio shown are for a typical experiment.

substance-P tracer to the same extent as did substance P itself (Fig. 2), both dose–response curves being superimposable. The remaining shorter fragments and eledoisin showed minimal cross-reactivity. The ^{125}I-labelled Tyr-substance P and physalemin tracers showed considerably lowered binding to both antisera. Compared with the [Tyr^8]-substance-P tracer (binding taken at 100%) binding of Tyr-substance-P tracer was 65%, whereas only 44% of physalemin tracer was bound by antibody. Since unlabelled physalemin cross-reacts 100% with our antisera, the presence of ^{125}I on the physalemin tracer may interfere with the antigenic recognition. Antigenic recognition is also affected by the addition of a tyrosine residue at the *N*-terminus (Tyr-substance P).

Since substance-P-(6–11)-hexapeptide is more potent on a molar basis than the undecapeptide, an antigenic recognition site must be located in this region. Physalemin contains the *C*-terminal amino acid sequence 7–11 in common with substance P. Eledoisin contains the *C*-terminal tripeptide in common with both substance P and physalemin. However, eledoisin displays minimal cross-reactivity with both antisera tested. It would seem, therefore, that the antigenic recognition site is contained within the region residues 6–8 of substance P, i.e. Gln-Phe-Phe. Since physalemin cross-reacts completely with substance P and contains a tyrosine residue at position 8 in place of the phenyl-

alanine residue in substance P, both amino acid combinations Phe-Phe or Phe-Tyr are suitable for antigenic recognition. Unlabelled [Tyr^8]-substance P exhibits the same affinity with both antisera as does the parent substance-P peptide. Fragments containing only the phenylalanine residue at position 7 and followed by a non-aramatic amino acid were not recognized, or recognized only slightly, by the antisera tested.

The *C*-terminal tripeptide and tetrapeptide have been shown to possess weak biological properties, whereas the *C*-terminal pentapeptides containing the antigenic recognition site for the two antisera tested produce appreciable biological activity. Sequences of six or more *C*-terminal amino acids have biological activity comparable with the parent undecapeptide (Bury & Mashford, 1976). Substitution of the phenylalanine residue at position 7 in the hexapeptide of physalemin produced a marked loss of the specific biological activity. Synthetic analogues with the tyrosine-8 residue replaced by leucine or alanine were practically devoid of activity (Erspamer & Melchiorri, 1973). Our antisera therefore appear to be directed against amino acids in positions 7 and 8 within the *C*-terminal hexapeptide fragment necessary for biological activity.

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Peroxidase Activity as a Possible Marker for a Functional Oestradiol Receptor in Human Breast Tumours

M. J. DUFFY and G. DUFFY

Department of Nuclear Medicine, St. Vincent's Hospital, Dublin 4, *Ireland, and Departments of Medicine and Surgery, University College, Dublin, Ireland*

Approx. 50–70% of human breast carcinomas contain a cytoplasmic oestradiol receptor. The presence of the oestradiol receptor is of considerable clinical significance, as slightly more than half of these oestradiol-receptor-positive tumours respond to either endocrine ablation or hormone administration (McGuire, 1975). The reason why the other 40–50% fail to respond is unknown. A possible explanation is that there is a failure in oestradiol action after its initial combination with the cytoplasmic receptor, i.e. there may be failure in the translocation of the cytoplasmic oestradiol receptor into the nucleus or a defect in nuclear binding. Therefore if instead of measuring the initial step in oestradiol action, one measured an end product of the hormone's action, such as an induced protein, a more accurate test of predicting hormone responsiveness might be available.

Oestradiol induces the synthesis of peroxidase both in the rat uterus (Lyttle & Jellinck, 1972; Anderson *et al.*, 1975) and in rat mammary tumours (Anderson *et al.*, 1975; DeSombre *et al.*, 1975). We therefore decided to investigate whether any relationship existed between the presence of oestradiol receptor and peroxidase activity in human breast tumours.

Human breast tumours were obtained from patients operated on in St. Vincent's Hospital. The oestradiol-receptor assay was carried out as previously described with the use of dextran/charcoal to separate free from bound oestradiol (Duffy & Duffy, 1977). Peroxidase activity was determined with guaiacol as substrate (McNabb & Jellinck, 1975). The reaction mixture contained 13mM-guaiacol, 0.3mM-H_2O_2, 10mM-Tris/HCl buffer, pH8.0, and 0.1 ml of cytosol in a final volume of 3 ml.

Table 1 compares the distribution of peroxidase activity in benign breast tumours and in both oestradiol-receptor-positive and -negative carcinomas. Only one out of 26 benign tumours, which were all without oestradiol receptor, contained any peroxidase activity. On the other hand, 78% of 37 oestradiol-receptor-positive carcinomas contained