*Acta virol.* **30**: 432—435, 1986

# SUBSTANCE P, A NEUROPEPTIDE, INHIBITS MEASLES VIRUS REPLICATION IN CELL CULTURE

C. SCHROEDER

Institut für Virologie, Bereich Medizin (Charité) der Humboldt-Universität, DDR — 1040 Berlin,
Institut für Virologie, Bereich Medizin (Charité) der Humboldt-Universität,
DDR — 1040 Berlin, G.D.R.

*Received December 17, 1985*

*Summary.* — Substance P, a neuropeptide of the tachykinin group, inhibits measles virus replication in cell culture and partially blocks viral fusion activity assayed in the haemolysis system. The $ID_{50}$ for the inhibition of measles virus single-cycle replication is 0.6 μmol/l, and the effect is fully reversible. The antiviral activity of substance P corresponds to that of previously described synthetic tri-to heptapeptides. Tachykinins and these oligopeptides share a short homology with the N-terminus of paramyxovirus fusion proteins.

*Key words*: *measles virus*; *antiviral activity*; *substance P*; *haemolysis*

Measles are still a major medical problem, causing 900 000 deaths annually (Mitchell *et al.*, 1985). Measles virus is neurotropic. The infection, and also the administration of live attenuated vaccine, are accompanied by reversible EEG changes. The infection can be complicated by various forms of encephalitis (Gendelman *et al.*, 1984). Vaccination programs, even in the developed world, have not succeeded in eliminating the virus (Mitchell *et al.*, 1985). Therefore, antiviral chemotherapy would be a useful supplement to vaccination.

Anti-measles compounds have been identified in vitro but have not been clinically tested. Oligopeptides (Norrby, 1971; Richardson *et al.*, 1980; Richardson and Choppin, 1983) and norakin (Presber *et al.*, 1984; Schroeder *et al.*, 1985) show promising activity in vitro. Measles virus-inhibiting peptides of 3—7 amino acids share the sequence phe-phe-gly with the N--terminus of the Sendai virus fusion (F1) protein. The peptides inhibit measles virus penetration by binding to sites on the cell surface which have been hypothesized to be F1 protein receptors (Richardson and Choppin, 1983). The sequence phe-phe-gly is also found in the neuropeptide substance P (for reviews see Porter and O'Connor, 1981; Oehme *et al.*, 1981) which consists of 11 amino acids: arg-pro-lys-pro-gln-gln-phe-phe-gly-leu-met-$NH_2$. Substance P belongs to a class of neuropeptides called tachykinins which

**Table 1. Inhibition of measles virus single-cycle reproduction by substance P**

| Substance µmol/l | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| | PFU/ml | % inhib. | PFU/ml | % inhib. |
| 0 | $7.6 \times 10^2$ | 0 | $4.06 \times 10^2$ | 0 |
| 0.0625 | $5.3 \times 10^2$ | 30 | n.d. | n.d. |
| 0.625 | $4.3 \times 10^2$ | 43 | $1.91 \times 10^2$ | 53 |
| 6.25 | $1.7 \times 10^2$ | 78 | $3.03 \times 10^1$ | 93 |
| 62.5 | 0 | 100 | $1.0 \times 10^1$ | 98 |

Vero cell monolayers in 10 ml scintillation vials were washed twice with serum-free medium and infected with serum-free measles virus at a MOI of 0.1 PFU/cell in the presence or absence of substance P. All further steps were performed in the presence of substance P, where indicated. The inoculum was removed after 1 hr incubation at 37 °C by washing once and incubation was continued at 37 °C for 23 hr. Background virus was assayed from control cultures harvested 2 hr p.i. Virus was harvested by three cycles of freezing and thawing and assayed as described (Presber *et al.*, 1984). Substance P was synthesized by M. Bienert (Institut für Wirkstofforschung der Akademie der Wissenschaften der D.D.R., Berlin).

differ (among others) in the central amino acid of phe-x-gly, x being phe, tyr, val or ile (Erspamer, 1981). The N-terminal sequence of measles virus F1 protein has recently been determined to be phe-ala-gly (Norrby, personal commun.).

In view of the sequence homologies between viral fusion proteins, inhibitory peptides and tachykinins, the effect of substance P on measles virus single--cycle reproduction was tested. Substance P inhibits single-cycle replication

**Table 2. Inhibition of measles virus-induced haemolysis by substance P, Boc-D-phe-L-phe-gly and Boc-L-phe-L-phe gly**

| Compound | µmol/l | % inhibition of haemolysis |
|---|---|---|
| Substance P | 6.25 | 8.5 |
| Boc-D-phe-L-phe-gly | 6.25 | 23 |
| Substance P | 62.5 | 32 |
| Boc-D-phe-L-phe-gly | 62.5 | 83 |
| Boc-L-phe-L-phe-gly | 100 | 23 |
| Boc-L-phe-L-phe-gly | 1000 | 67 |

*Cercopithecus aethiops* erythrocytes supplied in a stabilizing medium (2.5% glucose, 10 mmol/l citric acid, 45 mmol/l $Na_3$ citrate) by the Institut für Angewandte Virologie (Berlin) were used on the day of bleeding. The erythrocytes were washed 4—5 times with PBS at 4 °C. Erythrocytes were adjusted to 1% (vol/vol) final concentration in PBS at 4 °C, compound was added, followed immediately by serum-free measles virus concentrate, and the suspension was incubated for 30 min at 4 °C. Following a shift to 37 °C, incubation was continued for 2 hr in a shaker and terminated by rapid cooling in an ice bath. Erythrocytes were pelleted by centrifugation for 10 min at 1000 rev/min and released haemoglobin was determined spectrophotometrically. All assays were done in duplicate. The data were calculated from extinction differences between samples with and without (spontaneous haemolysis) virus. Average data from two independent experiments are given.

by more than 90% at 6.25 μmol/l, the $ID_{50}$ being about 0.6 μmol/l (Table 1). This activity is comparable to that of Z-D-phe-phe-gly in a plaque reduction assay (Richardson *et al.*, 1980). The antiviral effect of substance P is completely reversible: no inhibitory effect is observed in a multicycle reproduction assay, and in a plaque reduction test there is 50% inhibition, indenpendent of initial substance P concentration between 0.625 μmol/l and 62.5 μmol/l. In all three assays (single-cycle, multi-cycle and plaque reduction) with incubation periods from 1 to 5 days, the Vero cell monolayers revealed no toxic influence of substance P up to 62.5 μmol/l.

The mode of action of substance P as an inhibitor of measles virus replication is under study. Three, mutually not exclusive mechanisms are conceivable. An increase in cAMP levels (Robbins and Rapp, 1980; Miller and Carrigan, 1982) and interference with calmodulin-dependent enzymes in the presence of calcium (Bohn *et al.*, 1983) inhibit measles virus replication. The third, and more interesting possibility is that substance P, in analogy to the synthetic peptides, might block a cellular receptor for measles virus F1 protein.

Inhibition of measles virus fusion activity can be modeled in a haemolysis assay. The short oligopeptides have been shown to block measles virus-induced haemolysis (Norrby, 1971; Richardson *et al.*, 1980). Substance P also inhibits haemolysis (Table 2), however, markedly less than single-cycle reproduction (Table 1). Inhibition of haemolysis is observed at concentrations not below 6.25 μmol/l. The reference compound Boc-D-phe-phe-gly has a stronger, Boc-L-phe-phe-gly a weaker effect at these concentrations. Such a discrepancy between antiviral activities in virus reproduction assays and haemolysis tests was also observed in the case of Z-D-phe-phe-gly, where the respective $ID_{50}$s are 0.2 and 40 μmol/l (Richardson *et al.*, 1980).

The reversibility of the antiviral effect of substance P in cell culture and its relatively low activity in the haemolysis assay could result from proteolytic degradation of the neuropeptide. Substance P is attacked by various proteases (Kato *et al.*, 1978; Berger *et al.*, 1979; Chrétien *et al.*, 1980) and has an extremely short half life of about 15 sec in certain organs as in rat vas deerens, guinea pig ileum (Watson, 1983) or in the rat circulation (Conlon and Göke, 1984). We are presently investigating the effect of protease inhibitors on the antiviral and antifusion activities of substance P. These inhibitory activities could be due to substance P itself or to its degradation product(s) as has been suggested for specific physiological effects of the neuropeptide.

Whatever the antiviral mechanism of action of substance P is, its elucidation may contribute to our understanding fo the nature of the fusion protein receptor and of the molecular basis for measles virus neurotropism and persistence.

*Acknowledgements.* I would like to thank Dr. E. Gerike and Dr. A. Tischer (Institut für Angewandte Virologie, Berlin) for erythrocytes of *Cercopithecus aethiops*. The help of Dr. M. Bienert (Institut für Wirkstofforschung der Akademie der Wissenschaften der DDR, Berlin) in supplying substance P and the tripeptides, and his critical discussion of the manuscript are greatfully acknowledged. I am obliged to Dr. J. Langner (Institut für Physiologische chemie der Martin-Luther-Universität Halle-Wittenberg) for helpful suggestions.

## References


Berger, H., Fechner, K., Albrecht, E., and Niedrich, H. (1979): Substance P: In vitro inactivation by rat brain fractions and human plasma. *Biochem. Pharmacol.* **28**, 3173–3180.

Bohn, W., Rutter, G., Hohenberg, H., and Mannweiler, K. (1983): Inhibition of measles virus budding by phenothiazines. *Virology* **130**, 44–55.

Chrétien, M., Lee, C. M., Sandberg, B. E. B., Iversen, L. L., Boucher, R., Seidah, N. G., and Genest, J. (1980): Substrate specificity of the enzyme tonin: cleavage of substance P. *FEBS Lett.* **113**, 173–176.

Conlon, J. M., and Göke, B. (1984): Metabolism of substance P in human plasma and in the rat microcirculation. *J. Chromatogr.* **296**, 241–247.

Erspamer, V. (1981): The tachykinin peptide family. *Trends Neurosci.* **4**, 267–269.

Gendelman, H. E., Wolinsky, J. S., Johnson, R. T., Pressman, N. J., Pezeshkpour, G. H., and Boisset, G. F. (1984): Measles encephalomyelitis: Lack of evidence of viral infection in the central nervous system and quantitative study of the nature of demyelination. *Ann. Neurol.* **15**, 353–360.

Kato, T., Nagatsu, T., Fukasawa K., Harada, M., Nagatsu, I., and Sakakibara, S. (1978): Successive cleavage of N-terminal $arg^1$-$pro^2$ and $lys^3$-$pro^4$ from substance P but no release of $arg^1$-$pro^2$ from bradykinin by X-pro dipeptidylaminopeptidase. *Biochim. biophys. Acta* **525**, 417–422.

Miller, C. A., and Carrigna, D. R. (1982): Reversible repression and activation of measles virus infection in neural cells. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1629–1633.

Mitchell, C. D., and Balfour, H. H. (1985): Measles control: so near and yet so far. *Progr. med. Virol.* **31**, 1–42.

Norrby, E. (1971): The effect of carbobenzoxy tripeptides on the biological activity of measles virus. *Virology* **44**, 599–608.

Oehme, P., Bienert, M., Hecht, K., and Bergmann, J. (1981): Substanz P: Ausgewählte Probleme der Chemie, Biochemie, Pharmakologie und Pathophysiologie. Beiträge zur Wirkstofforschung 12, Institut für Wirkstofforschung, Berlin.

Presber, H. W., Schroeder, C., Hegenscheid, B., Heider, H., Reefschläger, J., and Rosenthal, H. A. (1984): Antiviral activity of norakin (triperiden) and related anticholinergic antiparkinsonism drugs. *Acta virol.* **28**, 501–507.

Richardson, C. D., and Choppin, P. W. (1983): Oligopeptides that specifically inhibit membrane fusion by paramyxoviruses: Studies on the site of action. *Virology* **131**, 518–532.

Richardson, C. D., Scheid, A., and Choppin, P. W. (1980): Inhibition of paramyxovirus and myxovirus replication by oligopeptides with amino acid sequences similar to those at the N-termini of F1 HA2 viral polypeptides. *Virology* **105**, 205–222.

Robbins, S. J., and Rapp, F. (1980): Inhibition of measles virus replication by cyclic AMP. *Virology* **106**, 317–326.

Schroeder, C., Heider, H., Hegenscheid, B., Schöffel, M., Bubovich, V. I., and Rosenthal, H. A. (1985): The anticholinergic anti-Parkinson drug norakin$^R$ selectively inhibits influenza virus replication. *Antiviral Res.* Suppl. **1**, 95–99.

Substance P in the Central Nervous System (R. Porter and M. O'Connor, Eds.) *Ciba Foundation Symposium* **91**, Pitman, London, 1981.

Watson, S. P. (1983): Rapid degradation of ($^3$H)-substance P in guinea pig ileum and rat vas deferens in vitro. *Br. J. Pharmacol.* **79**, 543–552.