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Lytic Action of Neurotropic Drugs on Retroviruses *in Vitro*

V. WUNDERLICH and G. SYDOW

Central Institute of Cancer Research, Academy of Sciences of the GDR, DDR-1115 Berlin-Buch, Lindenberger Weg 70/80, German Democratic Republic

Abstract—*Certain widely used nervous system drugs as chlorpromazine, promazine, haloperidol, propranolol and chlorphenethazine show a lytic action on retroviruses of both C-type and D-type* in vitro. *Some of the drugs also display moderate inhibitory activity on virion-associated reverse transcriptase.*

INTRODUCTION

WE HAVE demonstrated in recent studies [1, 2] that chelating agents like EDTA (ethylenediaminetetra-acetate) and EGTA (ethyleneglycol-*bis*-*N*,*N*'-tetra-acetic acid) have a lytic activity on certain retroviruses *in vitro*. This finding indicated a close association of divalent cations, presumably Ca^{2+}, with retroviral membranes. Since some nervous system drugs are able to displace Ca^{2+} from membranes [3], we were interested to examine the action of these agents on retroviruses. In this communication we report a strong lytic activity *in vitro* of some widely used neurotropic drugs on different retroviruses. This activity has been demonstrated by assaying the reverse transcriptase (RT) of isolated virus particles both before and after treatment with the agents.

MATERIALS AND METHODS

Drugs

The drugs listed in the Tables were obtained from a local pharmacy as commercially manufactured (eight different producers) solutions ready for injection. The solutions of different drugs were diluted 2–300-fold to the desired concentrations with 0.01 M Tris–HCl buffer (usually pH 8.3, in some cases pH 6.7 was needed to solubilize the drug completely). Crystalline tetracain was dissolved in the same buffer at pH 8.3.

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Viruses

Avian myeloblastosis virus (AMV) and Rauscher leukemia virus (RLV) were isolated from the plasma (kindly supplied by Dr. H. Liebscher and Prof. F. Fey) of leukemic chickens or NMRI mice, respectively. Primate type-D retroviruses used were isolated from virus-producing tissue culture cell lines: Mason–Pfizer virus (MPMV) [4] from human ovarial carcinoma cell line Tu 197 [5]; PMF virus (PMFV), a retrovirus released from malignant permanent human cells [5] which is closely related to but different from MPMV [6] also from Tu 197 cells; Squirrel monkey retrovirus (SMRV) [7] from human rhabdomyosarcoma cell line A 204. MPMV, SMRV, and the cell line A 204 were originally obtained from Dr. G. J. Todaro, NCI, by courtesy of Dr. J. Gruber, Office of Program Resources and Logistics, Viral Oncology, Bethesda, Md, U.S.A.

Virus particles were concentrated from plasma or 24 hr harvests of culture supernatants after clarification of cells and cell debris (14,000 ***g***, 15 min) by centrifugation (50,000 ***g***, 120 min) through a cushion of 30% sucrose (w/v) in TN buffer (0.01 M Tris–HCl, 0.1 M NaCl, pH 8.3). In some experiments, viruses were purified using both velocity sedimentation and isopycnic centrifugation in sucrose density gradients [8]. There was no influence of the degree of virus purity on the results.

Experimental procedure

Two different approaches have been used to demonstrate a lytic effect of the drugs on retroviruses.

(1) Virus (approximately 4 μg of protein) was resuspended in 20 μl Tris–HCl buffer, pH 8.3, containing 0.1 M dithiothreitol, and preincubated at 0°C for 30 min with an equal volume of a solution of either 2–5 mM drug or 0.1% Nonidet P 40 (NP 40). The RT reaction mixture (100 μl) was then completed and the assay was done as described [9].

(2) In another set of experiments, every virus preparation was divided into four aliquots assayed each for RT activity after treatment as follows:
   (a) virus not treated with drug and incubated without detergent;
   (b) virus not treated with drug and incubated with NP 40;
   (c) virus treated with drug as indicated and incubated without detergent;
   (d) virus treated with drug as indicated and incubated with NP 40.

Treatment of virus with drugs was performed by resuspending high-speed viral pellets thoroughly (about 4 μg of protein/ml) in 5 ml 0.01 M Tris–HCl buffer (pH 8.3 or 6.7) containing the drugs as indicated, followed immediately by pelleting at 50,000 ***g*** for 60 min at 4°C. Aliquots (a) and (b), serving as controls, were processed in the same way under omission of drugs. The final pellets were resuspended in 100 μl Tris–buffer, incubated at 0°C for 30 min with or without 0.05% NP 40, and tested for RT activity as described [9] using poly (rA).oligo (dT) (Boehringer) as a template. RLV was assayed in the presence of 0.3 mM $MnCl_2$ and the other viruses in the presence of 9.6 mM $MgCl_2$. The lytic activity (LA) of a given drug was defined as the difference of the amount of intact virus present before and after drug treatment referred to the initial amount of virus. LA was calculated according to the following formula:

$$LA = \left[1 - \frac{(d-c)}{(b-a)}\right] \times 100$$

where *a*, *b*, *c* and *d* denote the activities of RT (dis/min incorporated into acid-insoluble material) in each of the aliquots mentioned above. Incorporation of radioactivity in controls (b) was in the range of 50,000 to 200,000 dis/min. Release of RT activity in controls (a) was variable with different virus preparations but did not exceed a level of 10–15% of controls (b). Freshly harvested virus was preferentially used to obtain a level not higher than 5% of controls (b).

Drugs were tested for RT inhibitory activity in standard enzyme reactions by adding 10 μl of 5 mM drug solution (final concentration 1 mM) to 40 μl of NP 40- disrupted virus. Results were expressed as per cent of control without drug addition. Protein was assayed as described [10].

RESULTS

To evaluate the action of drugs two types of experiments were performed: (1) a direct approach using drugs instead of detergent in the preincubation step before the RT assay and (2) the experimental procedure for calculation of LA of a given drug as outlined in Material and Methods. The second, rather complicated approach was chosen to avoid difficulties arising from an inhibitory action of some drugs on RT activity as well as to increase the ratio of drug to viral protein.

As shown in Table 1, preincubation of SMRV with randomly selected neurotropic agents resulted in some release of RT activity from the viral particles. The release depended on the concentration of drug. Some of the drugs, however, exhibited an inhibitory effect on RT activity interfering with the enzyme assay. Therefore, in most experiments the second approach for evaluation of lytic action of drugs was used. Nevertheless, the inhibition of RT activity by a given agent (Table 2) did not correlate with its lytic activity. For unknown reason, the RT reaction of SMRV was found to be more susceptible to the drugs than the RT reaction of PMFV.

Table 3 demonstrates that representative neurotropic drugs are highly effective in disrupting retroviruses of both C- and D-type. Although some variations with the different viruses are observed, it appears that the drugs tested have similar lytic activities and are nearly as effective as detergents in lysing viruses. It should be noted that AMV, in contrast to its unresponsiveness to EDTA and EGTA [2], is also disintegrated by neurotropic drugs. As compared to chelating agents which even in high concentrations (up to 50 mM) only partially disrupt retroviruses [2], most of the drugs are able to completely disrupt virus particles in low millimolar concentrations. A survey of the effect of a variety of neurotropic drugs on PMFV is presented in Table 4. According to their different chemical structures the neuroleptics, which constitute the majority of the drugs under study, are

Table 1. *Release of* RT *from* SMRV *after preincubation with neurotropic drugs*

Virus sample	RT activity dis/min × 10^3	Percentage of totally lysed virus	Ratio of treated to untreated
Virus alone	25.2	9	1.0
Virus + 0.05% NP 40	280.6	100	11.1
Virus + 1 mM promazine	58.9	21	2.3
Virus + 1 mM haloperidol	58.9	21	2.3
Virus + 1 mM propranolol	53.3	19	2.1
Virus + 1 mM chlorphenethazine	101.0	36	4.0
Virus + 2.5 mM chlorphenethazine	171.1	61	6.8

Table 2. *Effect of neurotropic drugs on* RT *of type-*D *viruses*

Drug	Concentration (mM)	Percentage inhibition of RT of PMFV	SMRV
Chlorpromazine	1.0	44	not tested
Promazine	1.0	18	38
Haloperidol	1.0	27	51
Propranolol	1.0	17	36
Chlorphenethazine	1.0	9	30

Table 3. *Lysis of retroviruses by selected neurotropic drugs*

Drug	Concentration (mM)	LA values* AMV	RLV	PMFV	MPMV	SMRV
Chlorpromazine	0.2	60	94	47	68	49
	2.0	100	99	94		
	5.0		100	94	100	99
Promazine	1.0		65	76	97	100
	5.0			96		
Haloperidol	1.0	91	62	59	56	71
	5.0			90		
Chlorphenethazine	1.0	100	100	94	97	95
	5.0	100		95		
Propranolol	1.0	92	49	54	42	50
	2.5			90		

*Values were calculated from duplicate sets of enzyme activity determinations.

usually classified into four groups. Representatives of these groups display strong activity in disrupting PMFV. It may be of special interest that the local anaesthetics tetracaine which belongs to the drugs displacing very effectively Ca^{2+} from membranes [11] also exhibits high lytic activity, whereas its congener procain with its very limited ability to displace Ca^{2+} [11] has a relatively weak lytic activity.

results of the study presented here are consistent with our previous finding of a lytic action of certain chelating agents on retroviruses and support our conclusion that divalent cations, presumably Ca^{2+}, are closely associated with retroviral membranes [1, 2].

Since many other viruses also have a phospholipid-containing envelope the possibility exists, and has to be proven, that these viruses (including certain types of slow viruses

Table 4. Lytic Activity of various neurotropic drugs on PMFV

Class	Drug	Concentration (mM)	LA value*
Neuroleptics			
Phenothiazines	Chlorpromazine	5.0	94
	Promazine	5.0	96
	Butaperazine	5.0	95
	Methophenazine	1.0	99
	Fluphenazine hydrochloride	1.0	100
	Fluphenazine decanoate	5.0	93
Rauwolfia alkaloid	Reserpine	1.0	96
Butyrophenones	Haloperidol	5.0	90
	Trifluperidol	5.0	85
Dibenzodiazepine	Clozapine	5.0	93
Antiemetics	Chlorphenethazine	5.0	95
	Promethazine	5.0	81
Beta-adrenergic blocker	Propranolol	2.5	90
Local anaesthetics	Tetracain	5.0	70
	Procain	5.0	22

*Values were calculated from triplicate sets of enzyme activity determinations.

DISCUSSION

The neurotropic drugs used in this study, especially neuroleptics, are very soluble in biological membranes and fats and possess pronounced surface activity. Furthermore, it has been shown that their interaction with phospholipid constituents of biological membranes provokes displacement of loosely bound membrane Ca^{2+} by competing with it for negatively charged binding sites [3, 12]. Therefore, these agents may be regarded as detergent-like drugs and it is no surprise that they exert a strong lytic action on retroviruses as do detergents. As in the case of detergents, the mechanism by which neurotropic drugs cause disruption of retroviruses is not understood and has to be clarified in further studies. However, the above mentioned properties of the drugs probably contribute to their lytic activity. Despite the assumption that the surface activity of the drugs could play a major role in their lytic action on retroviruses, the possibly involved in causing some forms of chronic degenerative diseases like schizophrenia [13, 14]) are also susceptible to neurotropic drugs.

A further important question arising from our results is whether neurotropic drugs display an effect on retroviruses *in vivo*. *A priori* it cannot be predicted whether induced lysis is an activating or inactivating event in retrovirus life cycle. Our experiments demonstrate, however, that haloperidol, when administered *in vivo* in a similar drug to virus ratio as used in this study, both inhibits splenomegaly and prolongs mean survival time of mice infected with RLV [15]. Further studies to evaluate the efficacy of neurotropic drugs on retroviruses under *in vivo* conditions are in progress. Such studies may also be warranted because of some inhibitory effect of the drugs on RT activity *in vitro*.

Finally, the results of this study may add a new aspect to the long-continued but still highly controversial discussion on the relation-

ship between cancer incidence and schizophrenia in man (for a recent review see [16]). Since their introduction into medical practice neuroleptics have been widely used for long-term treatment of schizophrenic patients. This led to the hypothesis [17] that neuroleptic drugs like chlorpromazine are important factors in lowering cancer incidence among schizophrenics. However, no evidence for an association between breast cancer incidence and use of neuroleptics was found so far [18]. Nevertheless, as long as retroviruses may be considered as a cause of human cancer [19], the possibility should not be ruled out that neuroleptics interfere with some processes involved in virus-induced carcinogenesis in man. The present finding may be regarded as a first attempt to clarify this point. Clinical doses of at least some of the drugs, particularly if very high dose regimens (1000–2000 mg/day) are applied in both acute (chlorpromazine) and chronic (fluphenazine) schizophrenics (see [20]) or certain cardiac disorders (propranolol), result in molar drug concentrations in man that nearly come up to the concentrations shown in this study to be effective in disrupting retroviruses *in vitro*.

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REFERENCES

1. G. Sydow and V. Wunderlich, Demonstration of RNA-dependent DNA polymerase activity in non-disrupted type-D retrovirus particles after treatment with EDTA. *Acta biol. med. germ.* **36,** K53 (1977).
2. G. Sydow and V. Wunderlich, In preparation.
3. P. Seeman, The actions of nervous system drugs on cell membranes. In *Cell Membranes, Biochemistry, Cell Biology and Pathology.* (Edited by G. Weissmann and R. Clairborne) p. 239. HP Publishing, New York (1975).
4. E. M. Jensen, J. Zelljadt, H. C. Chopra and M. M. Mason, Isolation and propagation of a virus from a spontaneous mammary carcinoma of a rhesus monkey. *Cancer Res.* **30,** 2388 (1970).
5. A. Graffi, D. Bierwolf, R. Widmaier, E. Bender, V. Wunderlich, M. Rudolph, E. Mothes, A. Niezabitowski and G. Papsdorf, In der Gewebekultur züchtbares Oncorna-Virus in malignen permanenten Zellinien vom Embryo einer krebskranken Frau. *Dtsch. Gesundh.-Wes.* **29,** 1489 (1974).
6. B. Micheel, L. Baumbach, V. Wunderlich, A. Niezabitowski and D. Bierwolf, Serological evidence for antigenic differences between the Mason–Pfizer monkey virus (MPMV) and an MPMV-like virus (PMFV) detected in a malignant permanent human cell line. *Europ. J. Cancer* **15,** 101 (1979).
7. R. L. Heberling, S. T. Barker, S. S. Kalter, G. C. Smith and R. J. Helmke, Isolation of an oncornavirus from a Squirrel monkey (*Saimiri sciureus*) lung culture. *Science* **195,** 289 (1977).
8. C. G. Sciotti, M. Aikawa and R. R. Kohn, The evaluation of AKR leukemia virus purity: the requirement for both velocity and isopycnic centrifugation methods. *Analyt. Biochem.* **73,** 308 (1976).
9. V. Wunderlich, St. Zotter and G. Sydow, RNA-dependent DNA polymerase activity in intracytoplasmic type-A particles of mouse mammary tumors. *Acta biol. med. germ.* **35,** 1741 (1976).
10. G. Sydow, Eine einfache und schnelle Mikromethode zur Proteinbestimmung. *Acta biol. med. germ.* **38,** 519 (1979).
11. D. Papahadjopoulos, Studies on the mechanism of action of local anesthetics with phospholipid model membranes. *Biochem. biophys. Acta (Amst.)* **265,** 169 (1972).
12. P. Seeman, Anti-schizophrenic drugs—membrane receptor sites of action. *Biochem. Pharmacol.* **26,** 1741 (1977).
13. D. C. Gajdusek, The possible role of slow virus infection in chronic schizophrenic dementia. In *Neurochemical and Immunological Components in Schizophrenia.* (Edited by D. Bergma and A. L. Goldstein) p. 81. Alan R. Liss, New York (1978).
14. D. A. J. Tyrrell, R. P. Parry, T. J. Crow, E. Johnstone, I. N. Ferrier, Possible virus in schizophrenia and some neurological disorders. *Lancet* **i,** 839 (1979).
15. V. Wunderlich, F. Fey and G. Sydow, Antiviral effect of haloperidol on Rauscher murine leukemia virus. *Arch. Geschwulstforsch.* in press.

16. R. Martin du Pan and G. Müller, La mortalité par cancer chez les patients psychiatriques hospitalisés. *Schweiz. med. Wschr.* **107,** 597 (1977).
17. L. K. Csatary, Chlorpromazines and cancer. *Lancet* **ii,** 338 (1972).
18. S. Wagner and N. Mantel, Breast cancer at a psychiatric hospital before and after the introduction of neuroleptic agents. *Cancer Res.* **38,** 2703 (1978).
19. R. Hehlmann, RNA tumor viruses and human cancer. *Curr. Top. Microbiol. Immunol.* **73,** 142 (1976).
20. A. Rifkin, and F. Quitkin, What's new in the psychopharmacology of schizophrenia. *Bull. N.Y. Acad. Med.* **54,** 869 (1978).