Res. Virol.
1997, 148, 349-352

BRIEF NOTE

Mitogenic effect and activation of HIV1 production in serotonin-treated peripheral blood mononuclear cells derived from infected patients

S. Sidibé, F. Saal, N. Corvaïa, A. Rhodes-Feuillette, M. Canivet, J. Peries and L. Dianoux (*)

UPR A0043 CNRS, "Rétrovirus et Rétrotransposons des vertébrés", Bât INSERM, Hôpital Saint Louis, 75010 Paris

The regulation of the immune system is consi-dered, in general, to be dependent on a variety of different mechanisms and elements, i.e. antibodies, lymphokines, different subsets of T lymphocytes, B lymphocytes, macrophages etc. Independently of these intrinsic factors, insight has also been gained into the role of some extrinsic substances such as hormones and neurotransmitters, which can act as immune regulators. In fact, receptors for a large variety of hormones like glucagon (Bathena *et al.*, 1981), triiodothyronine (Csaba *et al.*, 1977), prostaglandin (Goodwin *et al.*, 1979) and neurotransmitters like β-adrenalin (Loveland *et al.*, 1981) are present in the lymphocytes. Moreover, the immunosuppressive activities of histamine have been well established (Ogden and Hill, 1980). More recently, Jackson *et al.* (1985) pointed out that serotonin (5-HT), a well defined neurotransmitter, can play a role in the immunological system; they suggested that serotonin modulation of the immune response occurs at the level of lymphocytes and macrophages.

In this context, we decided to study the relationship between 5-HT and HIV1-infected peripheral blood mononuclear cells (PBMC). Our interest was mainly to examine the possible effect of 5-HT on virus production. In this note, we present results concerning the existence of 5-HT mitogenic activity toward normal and HIV1-infected PBMCs and the capacity of the neurotransmitter to activate virus expression in the same type of cells cultured *in vitro*.

In a series of preliminary experiments, we studied the effect of physiological concentrations of 5-HT (10^{-6}M, 10^{-7} M and 10^{-8}) on *in vitro* proliferation of PBMCs from HIV1-infected patients and healthy controls.

After isolating PBMCs by Ficoll Hypaque centrifugation (Boyum, 1968) from 12 healthy subjects and 15 HIV1-infected patients, cells were incubated in 16-mm 96-well microplates at a density of 200,000 cells per well, in triplicate, in a total of 200 µl of growth medium per well (with 5% foetal calf serum (FCS)) in the absence or in the presence of various doses of 5-HT (dissolved in RPMI without serum, on ice, in darkness, 1 h before use). One row of four wells received, as mitogenic control, PHA alone at a final concentration of 5 µg/ml. The cells were incubated at 37°C in 5% CO_2 for 72 h. Twenty-four h before the end of incubation, 1 µCi of tritiated thymidine was added to each well. The cell cultures were harvested and thymidine incorporation was measured in a liquid scintillation counter and expressed as counts per minute (cpm).

Table I presents the results obtained. They are expressed as the stimulation index (SI) and show that, in most cases, 5-HT treatment at physiological concentrations induced a mitogenic response of PBMCs from infected and uninfected individuals.

Submitted August 20, 1996, accepted June 6, 1997.

(*) Corresponding author.

Table I. Effect of serotonin (5-HT) on PMBCs from healthy donors and HIV1-infected subjects.

Subject number	SI(*)			
	PHA	5-HT 10^{-8} M	5-HT 10^{-7} M	5-HT 10^{-6} M
Healthy subjects (n = 12):				
1	22.31	0.42	0.75	0.78
2	35.15	2.43	3.87	3.19
3	36.35	0.67	1.13	1.48
4	21.87	0.89	1.04	1.09
5	11.89	0.007	0.006	0.004
6	29.96	0.62	0.90	0.88
7	11.95	0.53	1.34	1.54
8	35.22	2.11	3.88	3.09
9	12.64	0.008	0.0085	0.0035
10	14.83	2.08	2.47	2.65
11	14.49	0.79	2.46	2.02
12	24.45	1.47	1.99	2.15
Asymptomatic HIV1 patients (n = 11):				
13	32.95	1.38	2.38	2.35
14	31.55	2.08	2.33	2.25
15	30.45	0.95	2.07	2.09
16	27.79	0.87	1.93	1.96
17	26.83	1.01	1.99	1.09
18	35.02	1.55	1.95	2.27
19	22.41	0.59	0.89	0.85
20	23.40	0.57	0.77	0.69
21	17.90	0.003	0.0015	0.0025
22	18.99	0.005	0.0067	0.0063
23	14.77	0.0012	0.003	0.0025
Symptomatic HIV1 patients (n = 4):				
24	28.08	1.30	3.85	3.45
25	25.86	1.95	3.95	3.25
26	12.10	0.0095	0.0090	0.0085
27	19.85	0.99	1.98	1.90
Patients with AIDS (n = 4):				
29	23.10	0.85	3.45	3.15
30	13.25	0.008	0.0085	0.0075
31	29.80	1.4	2.99	2.75
32	14.20	0.002	0.003	0.0015

(*) $SI = \dfrac{\text{mean cpm of stimulated sample} - \text{mean cpm of unstimulated sample}}{\text{mean cpm of unstimulated sample}}$.

The physiological concentrations of 5-HT stimulated DNA synthesis from 50 to more than 200% in 10 controls out of 12, in 8 asymptomatic subjects out of 11 and in 3 symptomatic patients out of 4. In 2 healthy subjects (nos. 5 and 9), in 3 asymptomatic subjects (2 were drug addicts; nos. 21, 22, 23) and 1 symptomatic patient (no. 26), 5-HT had not effect.

The mean of SI was 0.81 [0.0012-2.08] in the group of 19 patients and 1.002 [0.007-2.43] in the group of 12 healthy subjects ($p > 0.005$), for 10^{-8} M 5-HT.

The mean of SI was 1.60 [0.0015-3.95] in the group of 19 patients and 1.65 [0.0085-3.87] in the group of 12 healthy subjects ($p > 0.05$), for 10^{-7} M 5-HT.

The mean of SI was 1.47 [0.0015-3.45] in the group of 19 patients and 1.57 [0.0035-3.19] in the group of 12 healthy subjects ($p > 0.05$), for 10^{-6} M 5-HT.

cpm = counts per minute.
FCS = foetal calf serum.
5-HT = 5-hydroxytryptamine (serotonin).
PBMC = peripheral blood mononuclear cell.
PHA = phytohaemagglutinin.
SI = stimulation index.

By comparison with the untreated cell cultures, the activation of PBMC growth ranged from 50% to more than 200% ($p < 0.01$). This mitogenic effect was substantial, but weaker than that resulting from PHA treatment ($p < 0.001$). No mitogenic effect of 5-HT was observed on PBMCs of 2 control subjects, 3 asymptomatic subjects (2 of whom were drug addicts) and 1 symptomatic patient. Mean values of SI in infected patients and in healthy subjects indicate that the proliferation induced by 5-HT was not significantly different ($p > 0.05$).

In light of these results, we examined the effect of a 10^{-7}M 5-HT concentration on HIV1 activation in PBMCs obtained from 17 infected patients and 3 healthy controls. PBMCs were cultured in complete RPMI + 10% FCS, in 12-well plates (3 wells/assay) at 500,000 cells/4 ml/well, in the absence or in the presence of 10^{-7} M 5-HT. This treatment was renewed every 72 h. After 3, 6 and 10 days of culture, cell supernatants were collected, centrifuged at 1,500 *g* for 15 min and frozen at –80°C. Assay of p24 antigen was carried out on the cell supernatants using Abbott's test (Abbott Diagnostic, Rungis, France).

Quantification of the p24 viral protein in the supernatants of treated and untreated PBMC cultures is presented in table II. After 5-HT treat-

Table II. Quantification of HIV1 p24 protein (pg/ml) in PBMC cultures from patients, untreated or treated with 5-HT 10^{-7} M.

	3 days of culture		6 days of culture		10 days of culture	
Subject number	Control	+5-HT	Control	+5-HT	Control	+5-HT
Patients with AIDS (n=4):						
29	1073.5	1495.1(++)	1200.7	2967.2(++++)	1267.1	29992.7(++++)
30	848.9	855.1	850.3	857.2	854.5	856.6
31	1065.7	2098.7(+++)	1447.3	2879.1(+++)	1654.1(++)	2979.9(+++)
32	657.9	658.4	662.2	775.1	665.8	778.3
Symptomatic patients (n=5):						
24	1046.3	1180	1111.5	2275.5(++++)	1137.9(+)	2850.5(++++)
25	1042.1	2011.5(+++)	1230.2	2784.5(++++)	1542.1(++)	2989.5(+++)
26	1035.8	1195	1065.2	1190	1095.9	1195.1
27	1052	1062.5	1044.5	2611.9(++++)	1067.9(+)	2690.5(++++)
28	1038.5	1280.2(+)	1078.8	2098.7(+++)	1040.7(+)	2730.5(++++)
Asymptomatic patients (n=8):						
13	1085.2	1540.1(++)	1200.5	2947.2(++++)	1400.8	2975.5(++++)
15	805.2	1062.5(++)	1044.5	2611.9(++++)	1054.1(+)	2092.1(+++)
17	1020.2	1042.1	1095.3	1098.4	1102.1	1150.1
19	1135.1	1137.3	1110.1	1270.2	1140.2	1170.8
20	1015.2	1090	1095.2	1198.5	1100.8	2775.1(++++)
21	869.85	868.5	870.3	871.91	880.7	885.3
22	55.95	58.7	69.9	98.65	75.2	95.5
23	59.12	59.93	72.92	75.45	93.2	95.91
Healthy subjects (n=3):						
1	38	39	38	38.5	37	37.5
2	37	34.5	36	38	37.5	38
3	36	36.4	38	39.5	30.9	35

Antigen p24 was detected and quantified by the Abbott system in the supernatants of PMBC cultures (control or exposed at 5-HT 10^{-7} M) for 3, 6, and 10 days with postreatment every 72 h. The calculated concentrations of p24 (pg/ml) were an average of three independent experiments. In this test, the positive control used present a p24/p25 antigenicity around 1,000 pg/ml and the negative control used had a p24/p25 antigenicity equal to 37 pg/ml. In this system of detection, the values of p24/p25 equal to or superior to 1,000 pg/ml were considered as positive. The plus signs indicate the amplitude in p24 detection between control cells and 5-HT-treated cells (+ for weak stimulation to ++++ for strong stimulation (>100%)). After 5-HT treatment of the PBMCs, p24 expression was shown to be significantly increased in 9 patients out of 17.

ment at 10^{-7}M, p24 expression in cell culture supernatants was shown to be significantly increased in 9 patients out of 17. For the majority of positive cases, 2 to 3 treatments by 5-HT were necessary to obtain strong stimulation. The cells of each patient were controlled by PHA treatment at 5 µg/ml for 72 h. Viral production was positive in all cases (2,850 ± 840 pg/ml) except for two asymptomatic patients (numbers 17 and 19) and one patient with AIDS (number 32).

We demonstrate in this note that 5-HT at a concentration near the physiological range in peripheral blood exerts, in a statistical majority of cases, a mitogenic activity toward PBMCs from healthy and HIV1-infected individuals. Most importantly, we underline that 5-HT seems to activate HIV1 expression in infected PBMCs.

Stimulation of cell mitogenesis by 5-HT was previously described by Nemecek *et al.* (1986) in aortic smooth muscle cells. To our knowledge, our observation of a mitogenic effect induced by this neurotransmitter toward PBMCs is the first description of this phenomenon in cells implicated in the immunological response. The virus activation in PBMCs derived from HIV1 infected patients may be explained in part by this 5-HT mitogenic action.

Our results suggest that 5-HT may play an important role in the *in vivo* regulation of virus expression. Downmodifications in the level of the 5-HT concentration have been described in HIV1-infected individuals (Laplanche *et al.*, 1990; Launay *et al.*, 1989). Putting together these elements, it may be possible that upmodulation of 5-HT levels to above physiological concentrations, such as, for instance, in local acute inflammatory processes, could induce positive regulation of HIV1 production.

Key-words: Serotonin, HIV1; PBMC, Mitogenesis, Virus expression, Immunoregulation.

Effet mitogène et activation de la production de VIH1 dans les cellules mononucléées du sang périphérique provenant des patients infectés, traitées par la sérotonine

Dans le contexte du rôle éventuel de la sérotonine (5-HT) dans la régulation du système immun, nous avons examiné les effets de cette substance sur des cellules mononucléées du sang périphérique provenant d'individus sains et de patients infectés par le VIH1.

En premier lieu, nous avons constaté que la 5-HT à des doses correspondant à des concentrations physiologiques, peut exercer un effet mitogène. Il s'agit, à notre connaissance, de la première démonstration d'un effet stimulateur de la prolifération de cellules mononucléées sanguines par ce neurotransmetteur.

En second lieu, nous avons observé que le traitement des mêmes cellules par la 5-HT à la dose de 10^{-7}M donne lieu à une expression du VIH1 accrue, mesurée par le taux d'antigène spécifique p24, dans la majorité des échantillons cellulaires provenant d'individus infectés.

Mots-clés: Sérotonine, VIH1 ; CMSP, Mitogenèse, Expression virale, Immunoregulation.

References

Bathena, S.J., Louie, I., Schehter, G.P., Redman, R.S., Wahl, L. & Recant, L. (1981), Identification of human mononuclear leukocytes bearing receptors for somatostatin and glucagon. *Diabetes*, 30, 127-31.

Boyum, A. (1968), Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.*, 21, suppl. 97, 77-99.

Csaba, G., Sudar, F. & Dobozy, O. (1977), Triiodothyronine receptors in lymphocytes of newborn and adult rats. *Horm. Metab. Res.*, 9, 499-501.

Goodwin, J.S., Wiik, A., Lewis, M., Bankhurst, A.D. & Williams, R.C. (1979), High affinity binding sites for prostaglandin E on human lymphocytes. *Cell. Immunol.*, 43, 150-159.

Jackson, J.C., Cross, R.J., Walker, R.F., Markesbery, W.R., Brooks, W.J. & Roszman, T.L. (1985), Influence of serotonin on the immune response. *Immunology*, 54, 505-512.

Laplanche, S., Callebert, J., Prulière, D., Laplanche, J.L., Saal, F., Bolgert, F., Launay, J.M., Balli, J., Peries, J. & Dreux, C. (1990), Sérotoninémie, histaminémie et infection à VIH-1. *Act. Pharm. Biol. Clin.*, 5, 422-426.

Launay, J.M., Copel, L., Callebert, J., Corvaïa, N., Bricaire, F., Laplanche, J.L., Saal, F. & Peries, J. (1989), Serotonin and human immunodeficiency viruses. *Nouv. Rev. Fr. Hematol.*, 31, 159-161.

Loveland, B.E., Jannott, B. & McKenzie, I.F. (1981), The detection of beta adrenoreceptors on murine lymphocytes. *Int. J. Immunopharmacol.*, 3, 45-55.

Nemecek, G.M., Coughlin, S.R., Handley, D.A. & Moskowitz, M.A. (1986), Stimulation of aortic smooth muscle cell mitogenesis by serotonin. *Proc. Natl. Acad. Sci.* USA, 83, 674-678.

Ogden, B.E. & Hill, H.R. (1980), Histamine regulates lymphocyte mitogenic responses through activation of specific H1 and H2 histamine receptors. *Immunology*, 41, 107-114.