important to postulate that reexpression of GGT in proliferating precancerous foci and tumors of the liver is the result of a change in junctions between the cells and the extracellular matrix.

LITERATURE CITED

1. M. L. Anfimova and Yu. A. Rovenskii, Tsitologiya, 24, 1089 (1982).
2. Yu. Yu. Kusov and N.A. Kalinchuk, Analyt. Biochem., 88, 256 (1978).
3. S. M. Troyanovskii, Byull. Eksp. Biol. Med., No. 1, 81 (1982).
4. Z. Albert, J. Orlowski, M. Orlowski, et al., Acta Histochem., 25, 312 (1966).
5. J.Folkman and A. Moscona, Nature, 273, 345 (1978).
6. E. Huberman, R. Montesano, C. Drevon, et al., Cancer Res., 39, 269 (1979).
7. R. Montesano, G. Bannikov, C. Drevon, et al., Ann. New York Acad. Sci., 349, 323 (1980).
8. A. Novogrodsky, S. S. Tate, and A. Meister, Proc. Nat. Acad. Sci. USA, 73, 2414 (1976).
9. M. Orlowski and A. Szewczuk, Clin. Chim. Acta, 6, 430 (1961).
10. A. M. Rutenberg, H. Kim, J. W. Fischbeim, et al., J. Histochem. Cytochem., 17, 517 (1969).
11. B. Tsao and N. P. Curthoys, J. Biol. Chem., 255, 7708 (1980).

INHIBITORS OF SEROTONIN REUPTAKE AND SPECIFIC IMIPRAMINE BINDING IN HUMAN BLOOD PLASMA

O. S. Brusov, R. R. Lideman, A. M. Fomenko, and A. B. Katasonov

UDC 615.214.32.033.018.5+616.154.94:615.214.32]-074

KEY WORDS: serotonin; imipramine; endogenous imipramine-like ligands.

Many investigators have recently shown interest in the mechanism of the pharmacologic action of tricyclic antidepressants (TAD) in general and of imipramine (IMI) in particular. High-affinity binding sites of IMI and other TAD have been found in parts of the brain [6, 9, 10] and in platelets [11] of man and animals, of a decrease in the number of these sites in endogenous depression [2, 11, 12, 15], and of normalization of IMI binding in patients responding positively to treatment with TAD [8, 13], point to possible interaction between TAD and high-affinity binding sites of IMI, determining their antidepressant activity. The presence of specific IMI binding sites in brain tissue and at the periphery also suggests the existence of an endogenous imipramine-like ligand (or ligands) in man and animals, which may participate in the developmentof affective disorders in man [5, 7, 12].

In 1983 a low-molecular-weight endogenous ligand of IMI binding sites, capable of inhibiting binding of ^{3}H-IMI with its specific binding sites and serotonin (5-hydroxytryptamine, 5-HT) reuptake by rat brain synaptosomes [3], was discovered in brain tissue.

This paper describes a method of extraction of endogenous inhibitors of specific IMI binding and of 5-HT reuptake, from human blood plasma and the heterogeneity of these compounds is demonstrated.

EXPERIMENTAL METHOD

Blood (600 ml) from healthy donors (aged 25-30 years) was collected in plastic tubes containing anticoagulants (0.25 ml of 0.15 M EDTA to 10 ml of blood) and centrifuged at 200g for 20 min at room temperature. Platelet-enriched plasma was separated and centrifuged (10,000g, 10 min, 4°C). The plasma was collected and used for extraction, as described below. The platelet residue was washed twice (10,000g, 10 min, 4°C) in 66 ml of medium containing 50 mM Tris-HCl, 20 mM EDTA, 150 mM NaCl, pH 7.5. The residue was treated with an equal quan-

Laboratory of Clinical Biochemistry, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 26-28, July, 1985. Original article submitted June 4, 1984.

0007-4888/85/0007-0864$09.50

tity of 5 mM Tris-HCl, 5 mM EDTA, pH 7.5. The platelets were homogenized at 4°C and centrifuged at 20,000g (10 min, 4°C). The supernatant was removed and the membranes washed by centrifugation (20,000g, 10 min, 4°C) in 70 mM Tris-HCl, pH 7.5 (66 ml) and suspended in 40 ml of 50 mM Tris-HCl, pH 7.5, containing 5 mM KCl, 120 mM NaCl. To determine binding of IMI platelet membranes (1.5-2.0 mg protein) were incubated in a final volume of 0.6 ml, with 5 nM of ^{3}H-IMI and an aliquot of extract (0.2 ml) in 50 mM Tris-HCl, 5 mM KCl, 120 mM NaCl, pH 7.5, for 90 min at 0°C. The samples were then filtered through Mark GF/B filters and washed 4 times (5 ml each time) with cold incubation buffer. Specific binding was determined as the difference between binding of ^{3}H-IMI in the absence and in the presence of 50 μM IMI. Under these conditions specific binding amounted to 70-80% of total binding of ^{3}H-IMI [4].

To determine 5-HT reuptake, blood from healthy blood donors (30 ml) was collected in plastic tubes containing 1.5 ml of anticoagulant (7.6% sodium citrate) and centrifuged at 200g for 20 min at room temperature. The supernatant was collected and centrifuged at 10,000g (10 min, 4°C). The resulting platelet residue was suspended in 50 ml of medium containing 50 mM Tris-HCl, 5 mM KCl, 150 mM NaCl, and 0.38% sodium citrate, pH 7.4. The platelet suspension (0.8 ml) containing 0.35 mg protein in 1 ml was incubated in a final volume of 1 ml for 4 min at 37°C with ^{3}H-5-HT (200 nM) and aliquots of extract (0.2 ml) in medium of 50 mM Tris-HCl, 5 mM KCl, 150 mM NaCl, and 0.38% sodium citrate, pH 7.4, as described previously [1, 4]. The reaction was stopped by placing the samples in an ice bath, followed by filtering them rapidly through GF/B filters. The filters were washed 4 times with cold incubation buffer (5 ml each time), dried at 110°C, and counted on a "Mini-Beta" scintillation spectrometer (Finland), after addition of 7 ml of Lipolume scintillator (USA). Reuptake of 5-HT was determined as the difference between values of radioactivity of the filters obtained after incubation of the samples at 37 and 0°C.

The protein concentration was determined by a modified Lowry's method [14].

EXPERIMENTAL RESULTS

For extraction and partial purification of endogenous inhibitors of 5-HT reuptake and IMI binding 300 ml of fresh plasma was used. Alumina (60 g, type N super I, Woelm), washed with deionized water (5 times, 300 ml each time), was mixed in bulk with plasma and incubated with vigorous shaking at 0°C for 1 h. The mixture was centrifuged (2500g, 10 min, 2°C), the plasma was poured off, and the residue washed with 300 ml of cold deionized water. The mixture was again centrifuged under the same conditions and the residue washed with 300 ml of cold diethyl ether. The mixture was again centrifuged (2500g, 10 min, 2°C), the upper ethereal layer was poured off, and the alumina was incubated with vigorous mixing with 2 M acetic acid (160 ml) for 30 min at 0°C. The mixture was centrifuged (2500g, 10 min, 2°C), the supernatant was collected, and 160 ml of 2 M acetic acid was again added to the residue, and the mixture was incubated for 30 min (0°C) with vigorous shaking. The mixture was centrifuged under the same conditions and the pooled supernatant was evaporated at 25°C on a rotary evaporator (type RVO-64, Czechoslovakia). The residue was suspended in 40 ml of methanol and centrifuged at 45,000g (10 min, 4°C); the supernatant was dried at 25°C on a rotary evaporator. The dry residue was suspended in 30 ml water, titrated with a 2 M solution of tris-(hydroxymethyl)-aminomethane to pH 7.5, and centrifuged at 45,000g (10 min, 4°C). The supernatant was collected and filtered through a membrane ultrafilter of the YM-2 type (Amicon, USA), which passes only substances with a molecular weight of under 1 kilodalton. The filtrate was collected, lyophilized, and kept at −70°C until required for use. The total weight of extract was 1.17 g.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that extract obtained from human blood contains a material which inhibits dose-dependently both 5-HT reuptake and specific binding of ^{3}H-IMI. The maximal value of inhibition of 5-HT reuptake and IMI binding under these circumstances was 85%. Effective concentrations for inhibition of 5-HT reuptake and specific IMI binding (Fig. 1A, C) by the test extract were 0.36 ± 0.15 and 0.18 ± 0.1 mg/ml, respectively. Hill's coefficients for the inhibitory effect of the test extract on 5-HT reuptake and IMI binding were close to 1 (Fig. 1B, D). This is evidence of the absence of cooperative effects in the inhibition of 5-HT reuptake by platelets and of specific IMI binding by the extract.

To discover whether the inhibitory activity of the extract was due to the presence of one or several inhibitors in it, and also to purify the extract further, it was chromatographed on a column equilibrated with Biogel P-2 (Fig. 2). The experiments showed that the elution profile of the extract contained two minor peaks of inhibition of IMI binding and two princi-

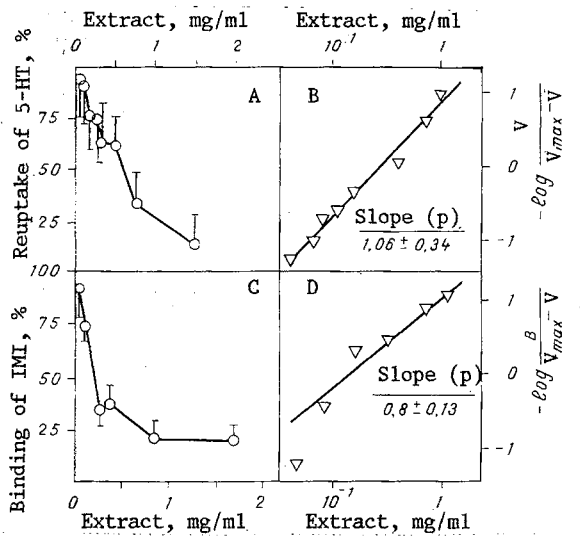

Fig. 1

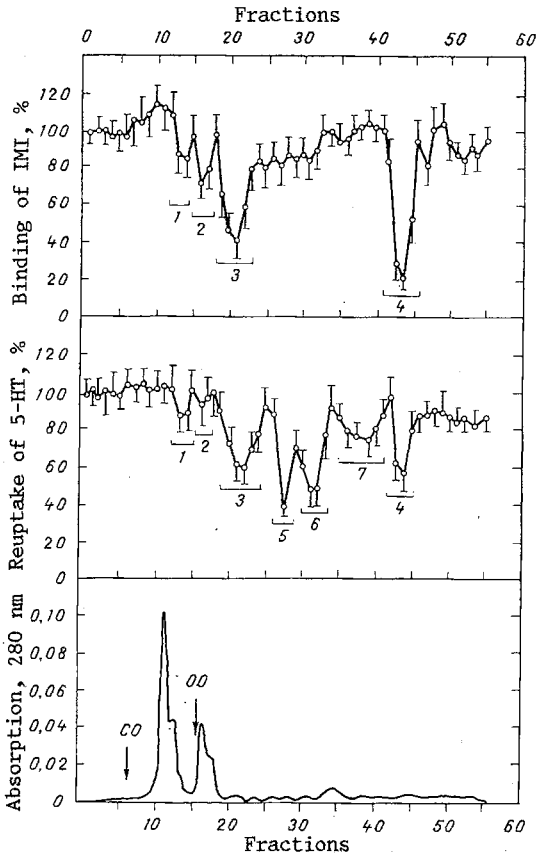

Fig.2

Fig. 1. Inhibition of reuptake of ^{3}H-5-HT and of specific binding of ^{3}H-IMI by extract of human blood plasma. Concentration of ^{3}H-5-HT in incubation mixture 200 nM, concentration of ^{3}H-IMI 5 nM. A, C) Results expressed in per cent of control level of ^{3}H-5-HT reuptake and of specific ^{3}H-IMI binding, respectively) B, D) results expressed in Hill's coordinates. V) Value (in cpm) of 5-HT reuptake (B) or of specific IMI binding (D) in presence of extract in incubation mixture. V_{max}) The same as V in absence of extract. p) Hill's coefficient. Straight lines drawn in B and D by the method of least squares.

Fig. 2. Gel-chromatography of extracts of human blood plasma on Biogel P-2. 500 mg of extract dissolved in 2 ml of deionized water and applied to a column (1.0 × 57 cm) with Biogel P-2 (400 mesh), equilibrated in deionized water; pH was adjusted with tris-(hydroxymethyl)-aminomethane to 7.5. Elution carried out with deionized water (pH 7.5) at 4°C at the rate of 4.5 ml/h. Fractions 3 ml in volume were collected. DV) Dead volume of column, OV) 1 volume of liquid in column.

pal peaks inhibiting this activity, which were eluted much later. The principal peaks inhibited reuptake of 5-HT by a much lesser degree than specific binding of IMI. Heterogeneity of the inhibitors of 5-HT reuptake also was discovered by chromatography of the extract on Biogel P-2 (Fig. 2B). Incidentally, under these circumstances three additional peaks of inhibition of 5-HT reuptake were discovered (peaks 5-7), with no marked ability to inhibit specific IMI binding. In addition, the principal peaks of inhibitory activity relative to IMI binding and 5-HT reuptake did not correspond to any of the well-marked peaks of absorption at 280 nm, evidence of the considerable additional purification of the inhibitors applied to the column. The principal peaks of inhibitory activity were eluted from the column after passage of at least one column volume on account of adsorption of the material on the Biogel P-2 matrix. This makes it impossible to determine the molecular weight of the extracted inhibitors by gel-chromatography on Biogel P-2.

LITERATURE CITED

1. L. Ahtee, M. Briley, R. Raisman, et al., Life Sci., 29, 2323 (1981).
2. B. Asarch, J. Shih, and A. Kulcsar, Commun. Psychopharmacol., 4, 425 (1981).

3. M. L. Barbaccia, O. Gandolfi, D.-M. Chuang, et al., Proc. Nat. Acad. Sci. USA, 80, 5134 (1983).
4. M. S. Briley, S. Z. Langer, R. Raisman, et al., Science, 209, 303 (1980).
5. E. Costa, D.-M. Chuang, M. L. Barbaccia, et al., Experientia, 39, 855 (1983).
6. A. Dubille-Ross, S. W. Tang, and D. Coscina, Life Sci., 29, 2049 (1981).
7. K. Fuxe, S.-O. Orgen, L. F. Aguati, et al., Neuropharmacology, 22, 389 (1983).
8. D. Healy, P. A. Carney, and B. E. Leonard, J. Psychiat. Res., 17, 251 (1982/83).
9. W. J. Kinnier, D.-M. Chuang, G. Gwynn, et al., Neuropharmacology, 20, 411 (1981).
10. S. Z. Langer, F. Javaj-Agid, R. Raisman, et al., J. Neurochem., 37, 267 (1981).
11. S. Z. Langer, E. Zarifian, M. Briley, et al., Life Sci., 29, 211 (1981).
12. S. Z. Langer and R. Raisman, Neuropharmacology, 22, 407 (1983).
13. H. Y. Meltzer, B. C. Arora, R. Baber, et al., Arch. Gen. Psychiat., 38, 1322 (1981).
14. G. L. Peterson, Analyt. Biochem., 83, 346 (1977).
15. J. L. Rausch, N. S. Shah, E. A. Burch, et al., Biol. Psychiat., 17, 121 (1982).

CHANGES IN THE BLOOD SYSTEM AFTER EXTRACORPOREAL IRRADIATION

Yu. I. Bobkov, V. Ya. Golikov', A. A. Belopol'skii, L. M. Alekseeva, L. A. Apollonova, S. S. Babayan, I. V. Burkov, and T. A. Vasina

UDC 612.1.014.482+615.38.
015.2:615.849.1

KEY WORDS: blood; irradiation; reactivity; immunity.

Whole-body irradiation of animals with comparatively small doses of radiation is known to be followed by an increase in the nonspecific resistance of the animal [8, 15], and by intensification of phagocytosis [3] and antibody formation [4]. The undesirable consequences of whole-body irradiation can be avoided by local and, in particular, by extracorporeal irradiation of blood (ECIB) [1, 9].

The aim of this investigation was to study the effect of a single ECIB on the cellular and biochemical composition of the blood and on activity of the factors of nonspecific immunity.

EXPERIMENTAL METHOD

Experiments were carried out on 16 mongrel dogs weighing from 12 to 28 kg. The animals were anesthetized by intravenous injection of a 2.5% solution of thiopental sodium and a bypass was created by means of a plastic tube between the femoral artery and vein. The development of thrombosis was prevented by injection of heparin in a dose of 200 U/kg body weight. The bypass tube was placed in a "Perenos" beta-apparatus, equipped with two IRUS-1 sources based on ^{90}Sr and ^{90}Y, with activities of $7.4 \cdot 10^{10}$ and $125.8 \cdot 10^{10}$ Bq. The blood was irradiated for 3 h to create a dose load of 6.0 to 12.0 Gy. The doses of irradiation of the blood were calculated by a mathematical method and by means of a chemical dosimetric system [5]. Blood was taken from a contralateral vein before formation of the bypass and in the course of 8 weeks after irradiation. The state of the blood system was assessed by reference to the following morphological and biochemical parameters: the erythrocyte and leukocyte counts, hemoglobin (Hb) concentration, hematocrit, ESR, activity of enzymes of the pentose phosphate pathway of glucose oxidation in erythrocytes (glucose-6-phosphate dehydrogenase (G6PDH) and transketolase) [12, 13], the concentrations of nucleic acids (DNA and RNA) [14], the total blood protein level (by Lowry's method), the concentrations of serum protein fractions, activity of factors of humoral and cellular nonspecific defense [6, 7, 10, 11], and also with reference to activity of benzylpenicillin, injected into the bloodstream in a dose of 20,000 U/kg body weight, by the agar diffusion method, using *Staphylococcus aureus* 209P as the test microorganism.

Central Research Laboratory and Department of Radiation Hygiene, Central Postgraduate Medical Institute, Moscow. Academic Group of Academician of the Academy of Medical Sciences of the USSR Professor Yu. F. Isakov. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. F. Isakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 28-31, July, 1985. Original article submitted September 14, 1984.

0007-4888/85/0007-0867$09.50