of free-radical formation in rabbit leukocytes was induced by $BaSO_4$ and registered in the presence of luminol as a chemiluminescent response [5]. We could reliably measure this reaction even though the amount of malonic dialdehyde formed was at least 10 times less than in previous experiments (Fig. 3). In these experiments carnosine effectively suppressed the oxidizing response of leukocytes, the native preparation being even more effective than the synthetic one.

The data presented show that the antioxidant activity of carnosine is governed by carnosine itself, the result of this action being not only local suppression of the chain LPO process, but also more intrinsic cell reactions, such as immunomodulating activity, stimulation of wound healing, stress adaptation, etc., - effects recently described [9]. At the same time, it is necessary to reinvestigate some effects of carnosine obtained with the use of commercial preparations in order to elucidate the contribution of the contaminations to the phenomena described.

REFERENCES

1. A. A. Boldyrev, E. C. Kurella, A. M. Rubtsov, *et al.*, *Biokhimiya*, **57**, № 9, 1360-1365 (1992).
2. A. A. Boldyrev, S. L. Stvolinskii, K. Pasqual, *et al.*, *Ibid.*, pp. 1337-1342.
3. A. M. Dupin, A. A. Boldyrev, Yu. V. Archipenko, *et al.*, *Byull. Eksp. Biol.*, **97**, № 8, 186-188 (1984).
4. A. R. Pavlov, A. A. Revina, A. M. Dupin, *et al.*, *Ibid.*, **110**, № 10, 391-393 (1990).
5. G. N. Semenkova, S. N. Cherenkevich, V. I. Levin, *et al.*, *Biofizika*, **30**, № 5, 921-922 (1985).
6. V. E. Formazyuk, T. Yu. Gorshkova, A. A. Boldyrev, and V. I. Sergienko, *Biochimiya*, **57**, № 9, 1324-1329 (1992).
7. L. V. Chasovnikova, V. E. Formazyuk, V. I. Sergienko, and Yu. A. Vladimirov, *Byull. Eksp. Biol.*, **104**, № 12, 668-670 (1989).
8. O. Aruoma, M. Lauchton, and B. Halliwell, Biochem. J., **264**, 863-869 (1989).
9. A. Boldyrev, A. Koldobski, E. Kurella, *et al.*, *Molec. Chem. Neuropath.*, **19**, (1993).
10. Ph. Hartman, Z. Hartman, and K. Ault, *Photochem. Photobiol.*, **51**, 59-66 (1990).
11. R. Kohen, Y. Yamamoto, K. Cundy, and B. Ames, *Proc. Nat. Acad. Sci. USA*, **85**, 3175-3179 (1988).

Effects of Steroid Hormones and Anti-Migraine Drugs on Serotonin Transport in Platelets of Patients Suffering From Migraine and in Those of Healthy Subjects

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Serotonin appears to play an important role in the pathogenesis of migraine. It has been established that during an attack of migraine the secretion of 5-hydroxyindoleacetic acid (the main serotonin metabolite) rises [9], while the serotonin level falls in platelets [2,4]. It is significant that migraine attacks are most often preceded by states characterized by elevated blood levels of steroid hormones (stress, emotional tension, use of contraceptives, etc.). Normally, steroid hormones probably do not participate in the regulation of serotonin transport in nerve endings or platelets [7,8]. Since increased levels of steroid hormones figure prominently among the factors triggering migraine attacks, the serotonin-transporting system of

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TABLE 1. Effect of Steroid Hormones and Drugs on ^{3}H–Serotonin Uptake by Platelets from Healthy Subjects. The Values are Percentages (Means±SEM) of the Respective Control Values

Concentration, mol/liter	Corticosterone	Hydrocortisone	Estradiol	Testosterone	Methysergide	Nicergoline	Tolfenamic acid	Flunarizine	Cinnarizine
5×10^{-9}	101±7.5	103±7.5	95±9.2	107±8.8	100±6.8	103±9.2	102±4.3	88±4.8	106±7.9
5×10^{-8}	99±8.6	98±7.4	104±8.4	97±6.8	103±8.1	91±7.1	101±3.5	103±7.0	107±7.9
5×10^{-7}	92±9.1	102±7.2	99±5.6	96±3.9	106±8.9	100±7.2	93±4.0	95±9.1	97±6.0
5×10^{-6}	102±5.4	98±4.6	104±4.6	101±3.8	94±4.6	99±6.8	91±4.4	61±9.7*	93±8.8
5×10^{-5}	102±8.9	93±7.0	53±2.7*	104±6.8	93±3.4	100±5.7	102±3.8	8±1.4*	24±1.2*
5×10^{-4}	NT	NT	NT	NT	63±4.4*	47±2.0*	102±9.8	0	0

Note. Here and in tables 2–4: NT = not tested; asterisk: $p<0.05$ by Student's t test.

migraine-sufferers may be presumed to have undergone substantial structural changes resulting in a high affinity of this system for steroid hormones. If so, then steroid hormones in such individuals may be thought to interact with the serotonin-transporting systems of platelets and nerve endings, thereby stimulating serotonin release during a migraine attack.

The purpose of this comparative study was to examine how steroid hormones affect serotonin uptake by and release from platelets in persons suffering from migraine and in healthy subjects. Some anti-migraine drugs capable of inhibiting serotonin uptake were also used for comparative purposes.

MATERIALS AND METHODS

To obtain platelets, venous blood was collected into a test tube containing a 3.8% sodium citrate solution (1/10 of the blood volume) and centrifuged at 150 *g* for 15 min. The resultant platelet-enriched plasma was again centrifuged at 650 *g* for 10 min. The isolated platelets were washed in buffer A (which contained 150 mmol/liter NaCl; 2.7 mmol/liter KCl; 0.37 mmol/liter NaH_2PO_4; 1.0 mmol/liter $MgCl_2$; 5 mmol/liter glucose; 10 mmol/liter HEPES-NaOH, pH 6.55; and 0.35% bovine serum albumin (BSA) fraction V and then resuspended in buffer B of the same composition as buffer A except that it contained 1.0 mmol/liter $CaCl_2$ and 10 mmol/liter HEPES-NaOH, pH 7.4. In each series of tests, pargyline was added to buffer B to a final concentration of 0.1 mmol/liter. To study platelets for their ability to take up ^{3}H-serotonin [3], 200 μl of a platelet suspension ($1\text{-}2\times10^8$ platelets per ml) were incubated in the absence (control tests) and presence of the drug concerned and of ^{3}H-serotonin (100 nmol/liter) for 20 min at 37°C and 0°C. Thereafter, the platelet suspension was passed through 0.45 μ Millipore filters which were then washed twice with a cold 0.9% NaCl solution. The content of radioactive material on the filters was measured by liquid radiometry. The specific uptake was determined as the difference between the uptake values obtained by incubating the platelet suspension at 37°C and 0°C. For the study of platelets for their ability to release serotonin [3], platelets were preincubated with ^{3}H-serotonin as described above. Thereafter, 200 μl of buffer B containing the drug concerned and 20 μl of preincubated platelets were incubated for 15 min at 37°C, after which the platelet suspension was passed through 0.45 μ Millipore filters which were then washed with a cold 0.9% NaCl solution. The content of radioactive material on the filters was also measured by liquid radiometry.

The following drugs were used: methysergide (Sandoz, Switzerland); nicergoline, cinnarizine, and flunarizine (NIKhFI, Bulgaria); and tolfenamic acid (Leiras Medica, Finland).

The patients were all males aged 30 to 45 years suffering from a simple form of migraine (migraine without aura). Blood samples were taken from them during periods when they were free of attacks. Healthy platelet donors aged 25 to 45 years were used as

TABLE 2. Effect of Steroid Hormones and Drugs on the Ability of Platelets from Healthy Subjects to Release ^{3}H–Serotonin. The Values are Percentages (Means±SEM) of the ^{3}H–Serotonin Amount Remaining in Preincubated Platelets after Their Incubation in the Absence of the Respective Compound

Concentration, mol/liter	Corticosterone	Hydrocortisone	Estradiol	Testosterone	Methysergide	Nicergoline	Tolfenamic acid	Flunarizine	Cinnarizine	Unlabeled serotonin
5×10^{-9}	104±1.4	102±2.1	101±2.2	104±2.0	NT	NT	NT	NT	NT	NT
5×10^{-8}	103±3.1	105±2.7	101±1.7	102±1.7	103±1.5	99±2.8	103±2.0	98±2.4	102±1.8	105±2.3
5×10^{-7}	101±3.8	99±3.2	102±1.8	103±2.8	103±2.3	101±2.2	105±2.1	98±2.9	107±2.6	105±1.8
5×10^{-6}	105±3.1	104±3.5	100±1.2	105±2.4	104±2.2	101±2.4	102±3.0	99±2.8	103±2.5	108±1.9
5×10^{-5}	104±1.1	104±1.8	98±1.2	102±1.9	101±2.0	101±2.9	102±3.1	69±6.0*	91±2.4	91±0.79*
5×10^{-4}	NT	NT	NT	NT	98±1.4	93±4.5	98±2.7	22±2.3*	27±3.1*	90±1.9*

controls. The results were treated statistically by Student's *t* test.

RESULTS

The results of tests to assess the effects of steroid hormones and drugs on ^{3}H-serotonin uptake by and release from the platelets of healthy subjects (controls) are summarized in Tables 1 and 2, respectively. As shown in Table 1, hydrocortisone, corticosterone, and testosterone did not influence appreciably serotonin uptake in the concentrations used. Flunarizine and cinnarizine inhibited its uptake markedly in concentrations of 5×10^{-6} and 5×10^{-5} mol/liter, respectively, while methysergide and nicergoline were markedly inhibitory in a concentration of 5×10^{-4} mol/liter. Estradiol inhibited serotonin uptake at 5×10^{-5} mol/liter. Tolfenamic acid did not affect serotonin uptake by platelets of normal subjects.

The results in Table 2 indicate that the compounds studied, with the exception of flunarizine and cinnarizine, had no effect on ^{3}H-serotonin release from the platelets of healthy subjects. Flunarizine and cinnarizine stimulated the release at 5×10^{-5} and 5×10^{-4} mol/liter, respectively. Unlabeled serotonin stimulated the release slightly: at 5×10^{-5} and 5×10^{-4} mol/liter it released not more than 10% of the labeled serotonin taken up by platelets.

The effects of the steroid hormones and drugs on the uptake of ^{3}H-serotonin by and its release from platelets of each patient are presented in Tables 3 and 4, respectively. Because the sensitivity of platelets to some of the compounds was altered in all patients in an individual manner, the data obtained in these tests could not be subjected to statistical treatment. (The data for compounds whose effects on ^{3}H-serotonin uptake by and release from patients' platelets did not differ from control values are omitted from these two tables.) As can be seen in Table 3, the serotonin-uptake system in four patients proved to be sensitive to tolfenamic acid: this inhibited considerably serotonin uptake by platelets from patients U and Kh. at 5×10^{-5} mol/liter and by those from patients P. and Sh. at 5×10^{-4} mol/liter. The concentration of cinnarizine required to inhibit serotonin uptake by platelets from all the patients was by one order of magnitude lower than in the control tests. Platelets from patient U. showed increased sensitivity to flunarizine: its concentration required to block serotonin uptake by his cells was one order of magnitude lower than in the control. Platelets from patients P and Z. exhibited a similarly increased sensitivity to estradiol. Corticosterone inhibited serotonin uptake by platelets from patients P., V., and L. at 5×10^{5} mol/liter. The effect of hydrocortisone on serotonin uptake by platelets from patient Sh. was surprising. It inhibited the uptake (by 76%) at 5×10^{8} mol/liter but was not inhibitory at higher concentrations.

As shown in Table 4, cinnarizine stimulated serotonin release from platelets of patients U., Kh., Sh., and Z. in concentrations that were one order of magnitude lower than in the control tests. The ^{3}H-serotonin-releasing effects of unlabeled serotonin were

TABLE 3. Effect of Steroid Hormones and Drugs on ^{3}H−Serotonin Uptake by Platelets from Patients with Migraine. The Values are Percentages (Means±SEM) of the Respective Control Values

Concentration, mol/liter	Patient U.			Patient Kh.			Patient P.		
	Tolfenamic acid	Flunarizine	Cinnarizine	Tolfenamic acid	Flunarizine	Cinnarizine	Cinnarizine	estradiol	corticosterone
5×10^{-9}	103	101	107	100	103	105	108	117	107
5×10^{-8}	91	95	97	97	109	93	82	115	100
5×10^{-7}	88	70	91	108	95	82	85	81	91
5×10^{-6}	93	25	54	90	31	36	42	64	113
5×10^{-5}	43	0	0	65	33	27	9	28	49
5×10^{-4}	16	0	0	40	32	21	6	NT	NT

Continue

Patient V.			Patient Sh.			Patient Z.		Patient L.	
Tolfenamic acid	Cinnarizine	corticosterone	Cinnarizine	nydrocortisone	Tolfenamic acid	Cinnarizine	estradiol	Cinnarizine	corticosterone
107	100	93	107	97	99	100	105	104	98
94	105	94	97	24	98	98	111	109	92
96	96	98	88	108	98	90	82	100	88
115	58	111	49	112	97	46	54	51	82
113	0	65	0	90	100	0	18	0	51
60	0	NT	0	NT	55	0	NT	0	NT

TABLE 4. Effect of Steroid Hormones and Drugs on the Ability of Platelets from Patients with Migraine to Release ^{3}H–Serotonin. The Values are Percentages (Means±SEM) of the ^{3}H–Serotonin Amount Remaining in Preincubated Platelets after Their Incubation in the Absence of the Respective Compound

Concentration, mol/liter	Patient U.	Patient Kh.					Patient P.		Patient Sh.	Patient Z.	Patient L.
	Cinnarizine	unlabeled serotonin	methysergine	Nicergoline	Tolfenamic acid	Cinnarizine	corticosterone	unlabeled serotonin	Cinnarizine	Cinnarizine	corticosterone
5×10^{-9}	–	–	–	–	–	–	102	–	–	–	101
5×10^{-8}	103	94	104	109	100	86	103	94	102	92	104
5×10^{-7}	102	80	107	108	85	93	95	95	102	92	98
5×10^{-6}	106	64	105	98	92	84	93	72	108	96	90
5×10^{-5}	54	58	96	99	92	22	70	80	21	30	65
5×10^{-4}	18	62	66	62	50	8	–	79	10	8	–

more strongly marked in tests with platelets of patients Kh. and P. Methysergide, tolfenamic acid, and nicergoline were capable of releasing considerable amounts of labeled serotonin from platelets of patient Kh. at 5×10^{4} mol/liter, while corticosterone showed a similarly high serotonin-releasing activity at 5×10^{5} mol/liter with platelets of patients P. and L.

In all patients, the systems mediating serotonin uptake and release displayed enhanced sensitivity to some of the compounds used. Since estradiol, methysergide, and nicergoline inhibit serotonin uptake by interacting with the binding site of serotonin translocase [1,7], the increased sensitivity of the serotonin uptake system in platelets of individuals suffering from migraine is likely to result from an increased affinity of that binding site for those three compounds. In several earlier studies, no substantial differences in the kinetics of serotonin uptake by platelets were noted between migraine sufferers and healthy controls [5,6], which suggests that the affinity of the serotonin translocase binding site for serotonin itself was not altered in the patients examined. One possible explanation for our present results, as well as those obtained previously, is that the structure of the serotonin-uptake system in the platelets of those who suffer from migraine is so altered that serotonin binding to the acceptor site of serotonin translocase remains normal and the uptake of serotonin itself is therefore not altered either.

The present study appears to support the hypothesis that structural alterations exist in the serotonin-transporting system of platelets in persons suffering from migraine. We, have not, however, been able to confirm the hypothesis that steroid hormones can, in physiological concentrations, impair serotonin transport and trigger a migraine attack in such persons. The increased sensitivity of platelets for steroid hormones in some of our patients was not sufficient to enable these hormones to interact with the serotonin-transporting system at physiological concentrations in those patients. Structural changes in the systems mediating serotonin uptake by and release from platelets may be one of the reasons for the involvement of platelets in the pathogenesis of migraine. Structural changes in the serotonin-transporting system may account for the enhanced sensitivity of this system to certain exogenous and endogenous compounds, including those that normally do not participate in the regulation of serotonin transport in platelets. As a result, such compounds may exert pathological effects on serotonin uptake by and release from platelets of individuals suffering from migraine and trigger serotonin release at the beginning of an attack. The fact that there is a similarity between the platelet serotonin uptake system and the serotonin uptake system of nerve endings [10,11] suggests that the serotonin-transporting system of nerve endings in patients suffering from migraine undergoes structural changes similar to those occurring in the serotonin-transporting system of their platelets.

REFERENCES

1. T. G. Pukhal'skaya, N. I. Maysov, and R. S. Mirsoyan, *Farmakol. Toksikol.*, № 6, 39-43 (1989).
2. M. Anthony, H. Hinterberger, and J. W. Lance, *Arch. Neurol.* (Chicago), **16**, 544-552 (1967).
3. J. L. Costa, D. L. Murphy, and H. Stark, *J. Physiol.* (London), **316**, 153-161 (1981).
4. D. A. Curran, H. Hinterberger, and J. W. Lance, *Brain*, **88**, 997-1010 (1965).
5. E. Hanington, R. J. Jones, J. A. J. Agmess, and B. Wachowicz, *Lancet*, **2**, 720-723 (1981).
6. O. Lingjarde and P. Monstad, *Cephalalgia*, **6**, 135-139 (1986).
7. M. C. Michel, A. Rother, C. Hiemke, and R. Ghraf, *Biochem. Pharmacol.*, **36**, 3175-3180 (1987).
8. M. Rehavi, H. Sepcuti, and A. Weizman, *Brain Res.*, **410**, 135-139 (1987).
9. F. Sicuteri, A. Testi, and B. Anselmi, *Int. Arch. Allergy Appl. Immunol.*, **19**, 55-58 (1961).
10. J. M. Sneddon, in: *Progress in Neurobiology* (G.A. Kerkut and A. Phillis, eds), Vol. 1, Pergamon Press, Oxford (1973), pp. 151-198.
11. S. M. Stahl, in: *The Platelets: Physiology and Pharmacology* (G. L. Longenecker, ed.), Academic Press, New York (1985), pp. 307-340.