with bicuculline suggests that GABA participates in the mechanism of the anticonflict action of dibunol.

The experimental results are evidence that the search for drugs with tranquilizing properties can be made in the antioxidant group and that their combined administration with tranquilizers may be indicated as a means of potentiating their anxiolytic effect while, at the same time, reducing their doses.

LITERATURE CITED

1. B. V. Andreev, G. E. Galust'yan, and I. V. Marusov, in: Minor Tranquilizers in the Treatment and Rehabilitation of Patients with Psychoneurologic and Psychosomatic Diseases [in Russian], Leningrad (1979), p. 20.
2. E. B. Burlakova, A. V. Alesenko, E. M. Molochkina, et al., in: Bioantioxidants in Radiation Damage and Malignant Growth [in Russian], Moscow (1975), p. 82.
3. E. B. Burlakova, in: Prospects in Bioorganic Chemistry for the Creation of New Therapeutic Agents [in Russian], Riga (1982), p. 186.
4. Yu. M. Vikhlyaev and T. A. Klygul', Zh. Nevropatol. Psikhiat., No. 1, 193 (1966).
5. Yu. A. Vladimirov and A. I. Archakov, Lipid Peroxidation in Biological Membranes [in Russian], Moscow (1972), p. 242.
6. F. Z. Meerson, Byull. Éksp. Biol. Med., No. 10, 404 (1979).
7. F. Z. Meerson, Adaptation, Stress, and Prophylaxis [in Russian], Moscow (1981), p. 92.
8. G. G. Neznamov, M. V. Poyurovskii, A. N. Kharlamov, et al., in: Pharmacology of Gamma-Aminobutyric Acid Derivatives [in Russian], Tartu (1983), p. 102.
9. L. D. Smirnov, T. A. Voronina, Yu. I. Vikhlyaev, et al., in: Physiologically Active Substances [in Russian], No. 10, Kiev (1978), p. 59.

EFFECT OF LONG-TERM PROPRANOLOL ADMINISTRATION ON SPECIFIC BINDING OF ^{3}H-WB-4101 WITH RAT MESENTERIC VASCULAR MEMBRANES

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UDC 615.217.24.033.136.4/.5

KEY WORDS: α_1-adrenoreceptors; mesenteric vessels; β-adrenoblockers.

Despite the ever widening application of β-adrenoblockers for the treatment of hypertension, the molecular principles of their therapeutic effect are still disputed [6]. Indirect evidence that prolonged administration of β-adrenoblockers can modify sensitivity of the α-adrenoreceptors of peripheral vessels to catecholamines has recently been published [4]. However, no direct indication of a change in specific binding of corresponding ^{3}H-ligands with vascular membranes during long-term administration of β-adrenoblockers could be found in the literature.

The aim of this investigation was, first, to study the affinity of certain β-adrenoblockers for specific binding sites of ^{3}H-WB-4101 (nowadays identifiable as α-adrenoreceptors) of brain membranes and, second, to study the characteristics of these same receptors in membranes of mesenteric vessels of rats during long-term administration of propranolol.

EXPERIMENTAL METHOD

Male Wistar rats weighing 180-200 g, kept in the animal house with water and food ad lib., and with natural alternation of daylight and darkness, were used. Unpurified synaptic membranes of the P_2 fraction were isolated from the brain by the method in [2] without modifications. The P_2 residue was next suspended in 0.05 M Tris-HCl buffer, containing 4 mM $CaCl_2$ (Tris-Ca), at the rate of 0.8-1.0 mg protein to 1 ml and kept at −20°C for not more than 1 week. To isolate

Institute of Pharmacology, Academy of Medical Scineces of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 7, pp. 38-40, July, 1985. Original article submitted August 31, 1984.

TABLE 1. Effect of Adrenoblockers on Specific Binding of ^{3}H-WB-4101 with Rat Brain Membranes (M ± m)

Compound	Concentration, M	Specific binding, % of control
L-propranolol	10^{-6}	100±9
	10^{-5}	80±10
DL-propranolol	10^{-6}	100±8
	10^{-5}	86±8
Nadolol	10^{-6}	100±5
	10^{-5}	100±6
Pindolol	10^{-6}	100±8
	10^{-5}	81±7
Atenolol	10^{-5}	100±6
	10^{-6}	81±6
Practolol	10^{-5}	100±9
	10^{-4}	100±8
Concentration, M	10^{-6}	100±7
	10^{-5}	73±7

Legend. Results of one typical experiment are given. Each value was measured in three repetitions. LB-1 is an original adrenoblocker synthesized at the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR.

TABLE 2. Effect of Long-Term Administration of Propranolol on Characteristics of Specific Binding of ^{3}H-WB-4101 by Rat Mesenteric Vascular Membranes (M ± m)

Parameter studied	Control	Experiment
Dissociation constant (K_d), nM	0,052±0,006	0,095±0,008*
Concentration of binding sites (B_{max}), femtomoles/mg protein	1,95±0,51	2,78±0,21

Legend. Results of three independent experiments given: each point obtained in three repetitions. *$P \leq 0.05$ in Student's t test. Values of K_d and B_{max} calculated by Scatchard's method.

the vascular membranes the rats were decapitated, the mesenteric vessels were carefully separated, freed of adipose tissue, and ground in a porcelain mortar with liquid nitrogen to the finely powdered state. This material was further processed like the brain tissue in [2] and the fraction sedimenting under the same conditions as fraction P_2 was isolated. The final suspension of membranes from mesenteric vessels was prepared and kept in the same way as the brain membranes. Propranolol was given to 14 animals for 15 days with their drinking water: the mean intake of the drug was 100 mg/kg per diem. Membranes were isolated from the vessels 24 h after stopping the drug. Specific binding of ^{3}H-WB-4101 was carried out by the method in [5], the only difference being that the final volume of incubation medium was 0.5 ml. Incubation continued for 30 min at 25°C, and was stopped on dilution of the samples with 8 volumes of cold Tris-Ca and rapid filtration in vacuo through GF/B glass fiber filters (Whatman, England). Filtration and washing the filters with the same buffer twice took not more than 30 sec. After drying in air, the filters were extracted in 5 ml of Bray's scintillator for 12 h. The radioactivity of the samples was determined on an Intertechnique SL-4000 scintillation counter (France). To study the affinity of the β-adrenoblockers 0.1 mM ^{3}H-WB-4101 was

used. Isotherms of specific binding of this ligand, because of the limited quantity of vascular membranes, were determined by the use of three concentrations of ^{3}H-WB-4101: 0.1, 0.5, and 1.0 nM.

As displacing agent 10^{-5} M phentolamine was used. The numerical results were subjected to statistical analysis by HP-33E microcalculator (USA). The protein concentration in the samples was determined by the method in [8].

EXPERIMENTAL RESULTS AND DISCUSSION

Some β-adrenoblockers have weak affinity for α-adrenoreceptors of brain synaptic membranes, exhibited only when these compounds are present in relatively high concentrations (Table 1). Nadolol and practolol, even in concentrations of 10^{-4} M, have no effect in general on specific binding of ^{3}H-WB-4101. These results indicate that the β-adrenoblockers studied have virtually no effect on α-adrenoreceptors. In other words, the quantity of β-adrenoblockers which may remain in biological membranes after long-term administration cannot evidently exert any significant effect on the state of the α-adrenoreceptors when their characteristics are studied in vitro.

The data in Table 2 show that administration of propranolol for 15 days led to a significant decrease in affinity of the α-adrenoreceptors for their specific antagonist WB-4101; their concentration (B_{max}) under these circumstances was not significantly changed. This fact sheds furtherlight on the mechanisms of the chronic effects of β-adrenoblockers on the character of α-adrenergic regulation of pressor vascular responses discovered previously [3, 4]. In the opinion of some clinicians, β-adrenoblockers do not lower the arterial pressure but rather prevent it from rising during physical exercise and emotional stress which, as we know, are accompanied by increased noradrenalin release. α-Adrenoreceptor blockade in this case can play an important protective role, protecting the cardiovascular system against possible complications and overloads [1, 9]. The possibility cannot be ruled out that desensitization of α_1-adrenoreceptors during long-term administration of propranolol, first revealed by the present writers, may reflect the presence of presynaptic negative self-regulation of noradrenalin secretion in adrenergic terminals of peripheral vessels. Blockade of presynaptic α-adrenoreceptors, which is virtually complete with the doses of propranolol used, may lead to a chronic increase in noradrenalin secretion. In turn, the increased noradrenalin concentration may be the cause of the observed desensitization of α-adrenoreceptors.

Despite the fact that the hypothesis given above is only one possible explanation of the mechanism of the observed α-adrenoreceptor desensitization, there is no doubt about the fact that a decrease in the sensitivity of these receptors, reducing the pressor response of the vessels to noradrenalin, may be an important factor in the realization of the hypotensive action of long-term administration of β-adrenoblockers.

LITERATURE CITED

1. G. A. Glezer, N. P. Moskalenko, and R. I. Megreleshvili, Kardiologiya, No. 3, 60 (1975).
2. V. V. Rozhanets, D. Yu. Rusakov, N. D. Danchev, et al., Byull. Éksp. Biol. Med., No. 7, 46 (1983).
3. F. W. Amann, P. Bolli, L. Hulthen, et al., Clin. Sci., 61, Suppl. 7, 1S (1981).
4. A. L. Draper, P. H. Rodbern, and A. T. Rogers, J. Pharm. Pharmacol., 120, 104 (1982).
5. D. A. Greenberg, D. C. U'Prichard, and S. H. Snyder, Life Sci., 19, 69 (1976).
6. A. J. Mann and A. D. Scalecamp, J. Cardiovasc. Pharmacol., 5, 530 (1983).
7. H. G. Morris, J. Allergy, 65, 2 (1980).
8. G. L. Peterson, Analyt. Biochem., 83, 346 (1977).
9. S. H. Tayler, C. Davidson, and W. Singleton, Clin. Sci., 50, 21 (1976).