EFFECT OF THE ANTIOXIDANT DIBUNOL, ALONE AND COMBINED WITH PHENAZEPAM, ON CONFLICT BEHAVIOR IN RATS

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KEY WORDS: phenazepam; antioxidant; dibunol; anticonflict effect; lipid peroxidation.

The role of stress in the etiology and pathogenesis of many nervous and mental disease is now generally accepted. There is experimental evidence that one step in the development of the stress syndrome is excessive lipid peroxidation (LPO) in cell membranes [2]. For example, more than 50% of the dry weight of substance in the brain consists of lipids and the intensity of LPO during emotional-painful stress (DPS) is approximately doubled [7]. Inhibitors of free-radical processes, in the form of natural and synthetic antioxidants, prevent activation of oxidation of membrane lipids developing in the internal organs during EPS, and thus exert a definite protective action against stress [8]. On the basis of the hypothesis that, by influencing the pathogenetic mechanisms of stress development, it is possible to act on its external (behavioral) and clinical manifestations, it was decided to study whether antioxidants possess any anxiolytic properties, considering that anxiety is an inevitable emotional component of all types of stress. There is experimental evidence that antioxidants of the 3-hydroxypyridine class have a moderately strong depressant effect on the CNS [10]. This was the reason for studying dibunol,* an antioxidant belonging to the group of screened phenols, which has found extensive application in clinical practice [6], in conflict situations, which constitute the most adequate model on which to assess the tranquilizing action of drugs [4]. The anticonflict action of dibunol and of the Soviet tranquilizer phenazepam (a bromazepam analog) has been compared. The effects of phenazepam and dibunol on this model were compared with the level of malonic dialdehyde (MDA), an end product of LPO, in the brain and peripheral blood. From the point of view of possible potentiation of the tranquilizing action of phenazepam, a combination of these substances has been studied.

Considering data on the role of gamma-aminobutyric acid (GABA) in the mechanism of action of tranquilizers of the benzodiazepine series and the fact that GABA-positive drugs have anxiolytic properties [9], the role of GABA in the mechanism of the effects of dibunol was studied in the investigation described below.

EXPERIMENTAL METHOD

The anticonflict effect of the compounds was studied on 130 noninbred male albino rats weighing 200-250 g. The animals were deprived of fluid to drink for 2 days. During the next 3 days the rats were kept in an experimental chamber with a drinking bowl for 10 min in order to form a water taking habit. On the 6th day the rats were placed twice in the chamber for 10 min each time [1]. On initial testing, 10 sec after the rats took water the first time, a direct current of 1 mA was applied to the feeding bowl, so that a conflict situation developed. After 2 h the test was repeated, and in this case the current was applied to the feeding bowl immediately after the animals had been placed in the experimental chamber. The number of times the rats drank water and their overall motor activity were recorded. Dibunol, phenazepam, and bicuculline (from Serva, West Germany) were used. Dibunol was administered *per os* in the form of a 20% oily solution, whereas phenazepam and bicuculline were injected intraperitoneally. All the drugs were given in a single dose, dibunol (80, 120, 240,

*4-Methyl-2,6-di-tert-butylphenol.

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and 400 mg/kg body weight) 90 min, phenazepam (0.1, 0.25, and 1 mg/kg) 30 min, and bicuculline (1 mg/kg) 10 min before the second test. Control animals received an equal volume of vegetable oil *per os*.

The effect of phenazepam and dibunol on LPO processes was judged by the change in MDA concentration, determined spectrophotometrically by the test with thiobarbituric acid [5], in the blood and brain tissue homogenate.

EXPERIMENTAL RESULTS

The results show that dibunol has an anticonflict action, which is dose-dependent (Fig. 1). This effect was not exhibited during chronic administration of the drug. The anticonflict action of dibunol is weaker than the corresponding action of phenazepam (Fig. 2). During their combined administration definite potentiation of the effects was observed, as shown by a marked increase in the number of times the animals took water. The antioxidant enabled the dose of the tranquilizer to be reduced by 75% without weakening its anticonflict action.

The tranquilizing effect of dibunol and of a combination of dibunol and phenazepam could be abolished by the GABA receptor blocker bicuculline.

As a result of the conflict situation LPO was activated by a marked degree after the first test and even more after the second test. During dibunol administration changes in the MDA level in the blood and brain tissues followed a similar course. Phenazepam, in doses of 0.1 and 0.25 mg/kg, lowered the MDA level, but by a lesser degree than dibunol. After combined administration of the drugs more marked inhibition of LPO was observed (Fig. 3).

The results thus indicate that dinbunol possesses anxiolotyc activity. Antioxidants, which act on LPO, are known to modify the phospholipid composition of cell membranes and their physicochemical properties (flowability, temperature of structural transformations, and so on) and, as a result of this, to modify activity of those enzymes and the sensitivity of those receptors whose effectors are phospholipids [3]. These facts can evidently explain the increased anticonflict effect of dibunol and phenazepam observed when they were administered together, as shown by an increase in the number of punishable visits to the drinking bowl. Antagonism

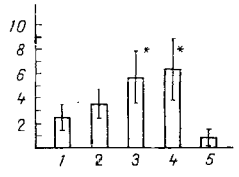

Fig. 1

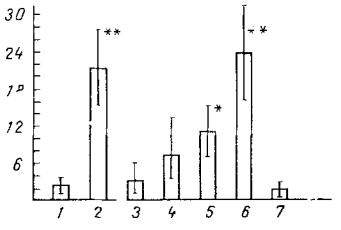

Fig. 2

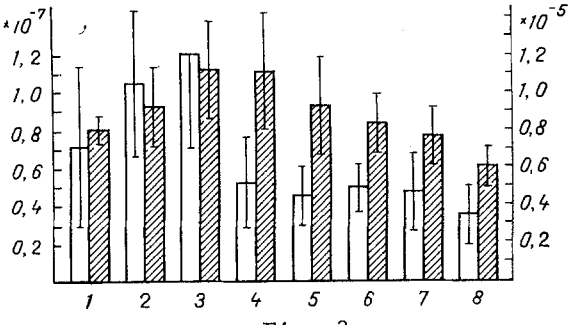

Fig. 3

Fig. 1. Effect of dibunol on index of tranquilizing action. 1) Control; 2-4) dibunol in doses of 80, 240, and 400 mg/kg, respectively; 5) dibunol (400 mg/kg) + bicuculline (1 mg/kg). Here and in Fig. 2: ordinate, number of times of taking water. *P < 0.05 compared with control.

Fig. 2. Effect of phenazepam and a combination of phenazepam with dibunol on number of times of taking water in conflict situation. 1) Control; 2-4) phenazepam in doses of 1, 0.1, and 0.25 mg/kg, respectively; 5) phenazepam (0.1 mg/kg) + dibunol (240 mg/kg); 6) phenazepam (0.25 mg/kg) + dibunol (240 mg/kg); 7) phenazepam (0.25 mg/kg) + dibunol (240 mg/kg) + bicuculline (1.0 mg/kg).

Fig. 3. MDA level in blood and brain of rats in conflict situation and changes produced by phenazepam and dibunol. Ordinate, MDA level (in μg/ml): unshaded columns (scale on left), in blood plasma ($\times 10^{-7}$); shaded columns (scale on right), in brain tissue homogenate ($\times 10^{-5}$). 1) Before creation of conflict situation; 2) after first conflict situation; 3) after second conflict situation; 4, 5) the same after injection of phenazepam in doses of 0.1 and 0.25 mg/kg, respectively; 6) the same after injection of dibunol (240 mg/kg); 7) the same after combined injection of phenazepam (0.1 mg/kg) and dibunol (240 mg/kg); 8) the same after combined administration of phenazepam (0.25 mg/kg) and dibunol (240 mg/kg).

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.

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

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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

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