BINDING OF ^{3}H-IMIPRAMINE BY PLATELETS OF SPONTANEOUSLY HYPERTENSIVE, NORMOTENSIVE, AND WISTAR RATS AND THEIR BEHAVIOR IN STRESS SITUATIONS

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A predisposition to psychopathological disorders is linked with individual psychoemotional reactivity to stress. The serotonin (5-HT) system also is implicated in the stress reaction. The role of genetic marker has been ascribed [16] to the characteristics of binding of ^{3}H-imipramine (^{3}H-IM) by platelets. as models of the 5-HT nerve terminals of the CNS [18], in biological concepts relating to the prediction of affective disorders. Binding sites of ^{3}H-IM on platelets and in the brain are associated with regions of 5-HT reuptake, but they are not identical [11]. The presence of an endogenous inhibitor of binding of ^{3}H-IM in the human and rat brain, blood plasma, and platelets [3, 13], by contrast with 5-HT, can modulate 5-HT reuptake [6] and, on account of this, can exert a significant influence on the psychoemotional state of individuals.

The aim of this investigation was to compare the characteristics of ^{3}H-IM binding by blood platelets of inbred lines of rats: spontaneously hypertensive (SHT), normotensive (NT) (Wistar-Kyoto rats), and Wistar rats with their behavioral manifestations in avoidable and unavoidable stress situations.

EXPERIMENTAL METHOD

Experiments were carried out on male SHT and NT rats (as the genetic control) and male Wistar rats weighing 250-300 g. The behavior of the animals in an avoidable stress situation was estimated by the extrapolation-dilution test (EAT) [1]. The latent period (LP) of motor activity, the number of unsuccessful attempts to escape, and LP of escape were recorded. The degree of depression of active defensive reactions of the animals in an unavoidable stress situation was assessed by Porsolt's test. The duration of immobile postures in the course of 10 min of the period while the rats were kept in water was recorded. Behavioral tests were carried out 2-3 days before the animals were sacrificed. Later, each of the tested rats (7-8 rats of each line) was lightly anesthetized with ether, after which 10 ml of whole blood was taken from the carotid artery into siliconized test tubes containing 0.38% of sodium citrate solution as anticoagulant. Platelet membranes were prepared and binding of ^{3}H-IM carried out by the method described previously [4]. Plateleten-riched plasma was twice washed with buffer. The platelets were fragmented and homogenized in hypotonic buffer and subsequently washed twice Specific binding of ^{3}H-IM (51.3 Ci/mmole, "NEN Chemicals"), was measured in the presence of 100 μM imipramine A suspension of membranes in three repetitions was incubated with ^{3}H-IM (1.2-9.6 mM) in a final volume of 300 μl for 60 min at 0°C, and after dilution, to 5 ml with cold buffer it was filtered through Whatman GF/F filters. The protein concentration in membrane homogenates (0.4-0.8 mg/ml) was determined by Lowry's method. Data on binding of ^{3}H-IM were subjected to Scatchard plot analysis. The results were read with coefficient of correlation of not less than 0.92. The results were subjected to statistical analysis by the Wilcoxon–Mann–Whitney test.

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TABLE 1. Values of Behavioral Parameters of SHT, NT, and Wistar Rats in Avoidable and Unavoidable Stress Situations

Line	EAT			Porsolt's test
	LP of motor activity	number of unsuccessful attempts at avoidance	LP of avoidance, sec	total duration of immobility, sec
NT (1)	3,3±0,9	0,9±0,03	8,4±1,7	130,1±20,7
SHT (2)	2,2±0,2	3,8±0,5	10,3±2,8	58.3±5,4
p_{1-2}	>0,05	<0,01	>0,05	<0,01
Wistar (3)	4,5±0,4	12,2±3,9	18.6±2,9	284,0±14,4
p_{1-3}	>0,05	<0,001	<0,001	<0,001
p_{2-3}	<0,05	<0,01	<0,01	<0,001

TABLE 2. Characteristics of Binding of ^{3}H-IM by Platelet Membranes of SHT, NT, and Wistar Rats

Line of rats	B_{max}, pmoles/mg protein	K_d, nM
NT	1,68±0,23	6,75±0,51
SHT	2,24±0,38	6,68±0,42
p_{1-2}	<0,05	>0,05
Wistar	3,25±0,25	4,72±0,25
p_{1-3}	<0,001	<0,05
p_{2-3}	<0,01	<0,05

EXPERIMENTAL RESULTS

The results relating to behavioral manifestations of the rats in avoidable and unavoidable stress situations are given in Table 1. Compared with SHT and NT rats, in Wistar rats in the EAT, LP of the motor responses was significantly increased, there was a marked increase in the number of unsuccessful attempts at avoidance (by 3.2 times compared with SHT and by 13 times compared with NT), and accordingly, LP of avoidance was increased This indicates that in these animals passive defensive reactions predominate over the active defensive type.

A specific feature of the avoidance behavior of the NT rats was the presence of a short period of immobility, as a result of which the making of the decision regarding avoidance was consequently considerably delayed (5 sec). Marked depression of active defensive responses in NT and Wistar rats was observed in an unavoidable stress situation. In Wistar rats the duration of immobility was 2.2 times longer than that for NT rats STH rats differed in the intensity of their active defensive reactions as early as when blood was taken from their hand Increased locomotor activity was observed in EAT, and it led to occasional mistakes but to rapid avoidance. In an unavoidable stress situation the duration of immobility was shortest of all, evidence of the lesser degree of proneness of SHT rats to depression of active-defensive reactions in stress situation.

It was found that 50% of the population of young SHT rats were unable to avoid in EAT. By contrast with young SHT rats, in which activity of tyrosine hydroxylase and dopamine-β-hydroxylase in the hypothalamus is increased, but the noradrenalin (NA) concentration was increased, and in adult SHR rats the values of these parameters in adult SHT rats in the absence of emotional stress were lower than those in adult rats [7, 20], 5-HT turnover also was depressed in the hypothalamus of adult SHT rats [10]. However, under conditions of emotional stress, activity of NA-synthesizing enzyme and release of 5-HT into the brain structures of adult rats, SHT rose sharply [7, 9]. In the hypothetical development of processes similar to denervation supersensitivity in the EAT suggested for adult SHT rats, an increase in regulation of postsynaptic hypothalamic receptors by 5-HT [10] and increasing release of monoamines in response to stress may lead to

strengthening of the response, namely to increased locomotor activity or to aggressiveness, without disturbing the cognitive component of activity.

A combination of difficulty of carrying out complex activity and of rapid development of immobility in Wistar rats in stress situations reflects their increased psychoemotional lability (rapid development of depression of adequate actively defensive reactions) compared with NT and SHT rats. The continuum of emotional-behavioral reactivity of the animals tested is thus represented by the following order: Wistar > NT > SHT. This is in agreement with data on the "fear" continuum of rats of these lines [19].

Characteristics of binding of ^{3}H-IM by the platelets of the animals studied are given in Table 2. The value of B_{max} was significantly reduced for NT rats compared with SHT. However, no differences were observed in the dissociation constant. In Wistar rats increased affinity and an increase in the number of binding sites of ^{3}H-IM were recorded compared with SHT and NT rats. Binding sites of ^{3}H-IM were associated anatomically and functionally with the 5-HT reuptake complex. It is claimed that in the oligomeric structure of the 5-HT-transport system one binding site — for tricyclic antidepressants, and another — for binding of HT and more specific blockers of 5-HT reuptake — may possess mutual allosteric effects [14]. It has also been found that a decrease in the 5-HT concentration in brain synaptosomes [17] and in the platelets of hooded rats leads to a decrease in H-IM binding [15]. In SHT rats the total pool of 5-HT-platelets is greater than in NT rats [8]. Evidently the difference in values of B_{max} in SHT and NT rats is connected with this fact. However, the decrease in the value of B_{max} in NT rats correlates with their greater predisposition to depressivelike reactions. These manifestations may be accompanied by the presence of an endogenous inhibitor of binding of ^{3}H-IM of nonpeptidergic nature in the blood and brain of NT rats. In that case, as was shown previously, the values of B for ^{3}H-IM are lowered [6]. A similar fall in the values of B_{max} is observed during chronic administration of IM [5]. In both cases the depressed regulation of ^{3}H-IM binding sites may depend on reduction of synthesis or internalization of these sites in the presence of the circulating inhibitor.

Increased emotional-behavioral reactivity of Wistar rats correlates directly with increased binding of ^{3}H-IM. The experiments showed that Wistar rats have increased sensitivity to stress, and that chronic emotional stress may lead to a twofold increase in binding of ^{3}H-IM by platelets in these animals [2]. Different mechanisms of modulation of ^{3}H-IM binding sites evidently exist and are linked with an allosterically controlled 5-HT reuptake complex in different strains of rats differing in their emotional-behavioral response in stress situations.

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