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# CORRELATION BETWEEN NUMBER OF TYPE 2 SEROTONIN RECEPTORS IN THE FRONTAL CORTEX AND INTENSITY OF SEROTONIN-INDUCED HEAD JERKS IN MICE

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Recent investigations have revealed two types of serotonin receptors in the brain: type 1 receptors (S1), binding $^3$H-serotonin specifically and with high affinity, and interacting with serotonin-sensitive adenylate kinase, and type 2 receptors (S2), highly sensitive to known blockers of serotonin receptors [11, 12, 14]. The functional role of the S1 and S2 receptors is not yet clear [5]. As regards the S2 receptors there is some evidence that they take part in some of the effects of serotonin: in serotonin-induced vasoconstriction of the rat caudal artery [12], in the hypothermic effect of serotonin [3], and in head jerking phenomenon [12, 14] which arises after injection of large doses of 5-hydroxytryptophan (5-HT) and which has been proposed as a test of functional activity of serotonin receptors [7, 13]. It has been shown that antagonists of S2 receptors prevent the development of this form of behavior [14].

Both to understand the functional role of S2 receptors and to assess the importance and informativeness of the head jerking test, it is necessary to know whether any connection exists between the number of S2 receptors and the intensity of this behavioral test. Correlation has recently been shown between the ability of various substances to block S2 receptors *in vitro* and their ability to inhibit the head jerking response in rats [12, 14]. In experiments with repeated electroconvulsions [9] and with chronic administration of antidepressants [6, 8] data have been obtained to show that changes in the number of S2 receptors were associated with changes in the intensity of head jerking. However, data on the character of the effect of antidepressants on the number of S2 receptors are contradictory [8]. Some definite light may be shed on this problem by investigations conducted on intact animals. Our attention was drawn to inbred lines of mice which, as we showed previously, differ in the functional state of the serotonin system of their brain: activity of tryptophan hydroxylase [2] and the concentrations of serotonin and its principal metabolite 5-hydroxyindoleacetic acid [1].

In the present investigation interlinear differences were discovered in the number of S2 receptors in the cerebral cortex and they were compared with the intensity of 5-HT-induced head jerking in mice of inbred lines.

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TABLE 1. Number of S2 Receptors in Frontal Cortex of Inbred Mice and Intensity of Serotonin-Induced Head Jerking (M ± m)

| Line of mice | Number of jerks | Number of S2 receptors, femtomoles/mg |
|---|---|---|
| BALB/c | 20,5±2,6 (11) | 36,9±4,0 |
| DBA/1 | 26,4±6,8 (9) | 37,6±3,5 |
| C3H/He | 53,4±10,0 (9) | 35,2±3,7 |
| C57BL/6 | 53,8±5,9 (10) | 34,8±1,9 |
| AKR | 111,1±10,8 (8) | 46,2±3,4 |
| CBA | 155,0±14,7 (6) | 53,0±2,4 |
| CC57Br | 185,6±24,0 (7) | 59,6±3,9 |

Legend. Number of experiments given in parentheses. Number of S2 receptors was measured in five animals of each line.

## EXPERIMENTAL METHOD

Experiments were carried out on mature male mice of seven inbred lines: BALB/c, AKR/J, CBA, CC57Br, C3H/He, C57BL/6, DBA/1. All the animals were aged 2-3 months and weighed 20-30 g. The mice were kept under standard animal house conditions. Immediately before the experiments the animals were put out into separate cages, in order to abolish any group effect. Head jerking was induced in the mice by injection of the serotonin precursor 5-HT intraperitoneally in a dose of 200 mg/kg. Characteristic irregular stereotyped head jerking movements began to be recorded 10 min after injection of the compound, and they continued to be counted for 20 min.

The number of S2 receptors was estimated from the quantity of $^3$H-spiperone (specific radioactivity 17 Ci/mmole, concentration in medium 0.8 nM, from Amersham Corporation, England), which is specifically bound by brain membrane preparations. The mice were decapitated, the brain was quickly removed in the cold, and the frontal cortex was separated. Spiperone is known to be mainly bound with S2 receptors in this region of the brain [14]. The number of receptors was determined by a method which was a modification of that described previously [15]. To reduce nonspecific binding of $^3$H-spiperone, incubation took place in a solution containing 0.05 M Tris-HCl, pH 7.7, 5 mM KCl, 0.12 M NaCl, 2 mM $CaCl_2$, $10^{-5}$ M pargyline (from Serva, West Germany), and 0.1% ascorbic acid. The spiperone concentration was 0.8 M. Specific binding was assessed as the difference between the quantity of label bound in the absence and in the presence of methylsergide (from Sandoz, Switzerland), a drug which specifically blocks S2 receptors, in a concentration of $10^{-6}$ M, and expressed in femtomoles $^3$H-spiperone/mg protein. Protein was determined by Lowry's method. The coefficient of correlation was calculated and its significance estimated by the standard method.

## EXPERIMENTAL RESULTS

The head jerking response to injection of a large dose of 5-HT is manifested as a characteristic rapid and abrupt movement of the head, analogous to reflex twitching in response to stimulation of the external auditory meatus with a hair. In all lines of mice studied, this response to injection of 200 mg/kg of 5-HT has been shown to be exhibited quite clearly (Table 1), so that there is no need to give simultaneously an injection of peripheral blockers of decarboxylase of aromatic amino acids, as some invesitgators have suggested [13]. Meanwhile mice of the lines studied differed significantly in the intensity of this form of behavior. In mice of lines most sensitive to activation of the serotonin system (AKR, CBA, CC57Br) the number of jerks was seven or eight times greater than the number of jerks observed in animals of lines BALB/c and DBA/1. Differences between the lines were significant and substantially greater than differences within the lines ($F = 38.5$; $P < 0.01$).

Significant interlinear differences, although less marked, were observed when the number of S2 receptors was determined in the frontal cortex of the mice. Maximal differences in the number of S2 receptors in mice of the seven lines studied were almost twofold (Table 1). Significant dependence of the number of S2 receptors in the frontal cortex on genotype was established by dispersion analysis. Interlinear differences, determined by the animals' genotype, were significantly greater than intralinear differences, associated with the effect of the external environment ($F = 8.6$; $P < 0.01$). The greatest number of S2 receptors was ob-

served in animals of lines CC57Br, CBA, and AKR, i.e., in those lines in which the response of head jerking to injection of 5-HT was most marked.

The coefficient of interlinear correlation between the number of head jerkings in animals of different lines and the number of S2 receptors in the frontal cortex of mice of these same lines was extremely high ($r = 0.96$; $P < 0.001$). This is evidence of close positive genotypic correlation between these parameters.

This fact would appear to be important in principle, because it provides the answer to one of the main questions, namely, does the labeled preparation bind with the functionally active receptor, or is this binding due to interaction with an acceptor [10]. The most important evidence as yet that the ligand binds with the receptor was the discovery of correlation between the ability of drugs to displace this ligand in tests *in vitro* and to block the receptor in tests *in vivo* [10, 11, 14]. However, even the presence of this correlation is not a complete guarantee that this is receptor binding [10]. The high positive interlinear correlation established between the number of receptors and the intensity of the head jerking response is proof that $^3$H-spiperone does in fact bind in the frontal cortex of mice with functionally active serotonin receptors. Since $^3$H-spiperone binds in the frontal cortex mainly with S2 receptors [14], the presence of correlation is further evidence that 5-HT-induced head jerking is due to activation of S2 receptors. Correlation between the number of S2 receptors and the number of head jerks means that this simple test can be used for the rapid assessment of the relative number of S2 receptors in the brain.

The presence of high correlation between the number of S2 receptors and the number of head jerkings in mice reflects a genetic connection between this form of behavior and the serotonin system, and it in no way signifies that this phenomenon is controlled purely by the serotonin system and cannot be modulated by other mediator systems, for example, the adrenergic system, as Shchelkunov [4] has demonstrated.

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