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Similarity of 5-HT2 Receptor Sites in Dominant and Subordinate Vervet Monkeys

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BRAMMER, G. L., M. T. McGUIRE AND M. J. RALEIGH. *Similarity of 5-HT2 receptor sites in dominant and subordinate vervet monkeys.* PHARMACOL BIOCHEM BEHAV **27**(4) 701–705, 1987.—Pharmacological studies using serotonergic agents have revealed status-linked behavioral effects in dominant and subordinate vervet monkeys. A possible explanation for the greater drug response observed in dominant animals is that there is a CNS difference between dominant and subordinate animals. Such differences could exist at the level of serotonin receptor sites, membrane responsiveness, or interaction with other neurotransmitters. We have examined the specific 3H-ketanserin binding in various regions of vervet monkey brain to evaluate the hypothesis that dominant and subordinate vervet monkeys differ in CNS 5-HT2 receptor sites. No differences were found in the number or affinity of 3H-ketanserin binding sites between dominant and subordinate animals. Further, no differences were found in the displacement of 3H-ketanserin binding by the serotonin agonist quipazine. These results suggest the conclusion that differences at 5-HT2 binding sites do not account for status-linked differences in behavioral drug response in vervet monkeys and that other or additional mechanisms must underlie status-related drug response differences.

Vervet monkey Serotonin Ketanserin Quipazine 5-HT2 receptors

WE have previously described physiological and behavioral differences between adult male vervet monkeys *(Cercopithecus aethiops sabaeus)* of different social status. The initial parameter reported to differ was whole blood serotonin (WBS) [11]. Behaviorally dominant males had higher WBS concentrations (about 1000 ng/ml) than subordinate males whose WBS levels were typically about 600 ng/ml. Further, differentiated WBS levels were found to be associated with social status rather than being a characteristic trait of individual animals. When animals changed social status, either spontaneously or as a result of experimental manipulation, their WBS levels changed accordingly. Dominant and subordinate animals also differed in their responses to individual behavior test situations such as exploration of novel objects and response to mildly threatening stimuli [8].

Drug challenge studies revealed status-linked differences in physiological and behavioral responses. For example, relative to subordinate males, dominant males responded to tryptophan load with a greater absolute and relative increase in WBS [2] and also with a larger change on several behavioral measures [12]. The serotonin uptake inhibitor 3-(p-trifluoro-methylphenoxy)-N-methyl-3-phenylpropylamine (fluoxetine) and the serotonin receptor agonist 2-(l-piperazinyl)quinoline (quipazine) elicited status-related behavioral effects similar to tryptophan [12]. All three drugs, tryptophan, fluoxetine, and quipazine, produced dose-dependent increases in approaching, grooming, resting, and eating, decreases in locomoting, avoiding, being vigilant, and being solitary, and had minimal effect on the occurrences of other behaviors including aggression and sexual behavior. Furthermore, 6 of the 8 behaviors consistently altered by tryptophan, fluoxetine, and quipazine also exhibited a status-interaction. For these 6 serotonin-influenced behaviors, dominant animals were more responsive to drug treatment than were the subordinate animals. The greater drug sensitivity of the dominant animal was observed both in behaviors that were increased as well as those that were decreased in rate by the drug treatment. If the relevant actions of tryptophan, fluoxetine, and quipazine were, respectively, to stimulate serotonin synthesis, block serotonin reuptake, and occupy serotonin receptors, it seems unlikely that differences in drug metabolism or transport could account for the observed behavioral response differences. An alternate explanation for the interaction between status and drug response is that the central nervous systems of dominant and subordinate animals differ in their responsiveness to drugs.

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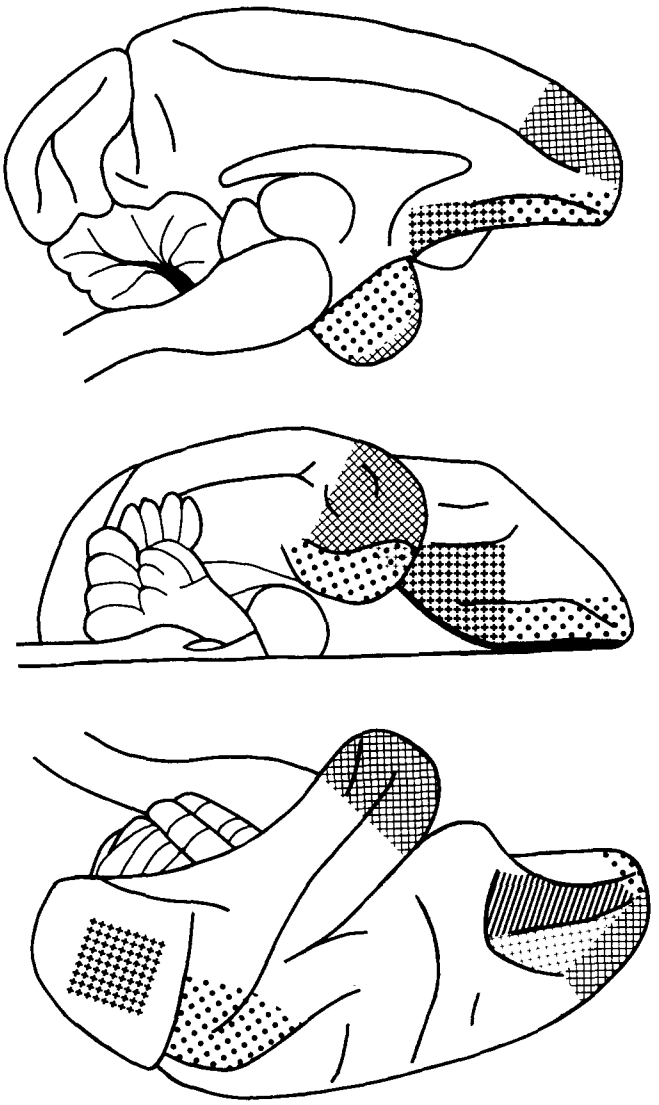

FIG. 1. Cortical brain areas sampled. Medial, ventral, and lateral views of vervet monkey brain are presented. These drawings were prepared with reference to *in situ* fixed brain and depict fresh tissue approximately. The sampled areas of medial and inferior frontal cortex, medial/inferior parietal cortex, and occipital cortex are visible only in the lateral view. The sampled areas of posterior orbitofrontal cortex and uncus (including amygdala) are visible in both medial and ventral views, and the sampled area of superior frontal cortex is visible in both lateral and medial views. Sampled areas of temporal tip cortex and orbitofrontal cortex are visible in all three views.

If tryptophan, fluoxetine, and quipazine acted as indicated above, an early locus of action common to the three drug treatments would be stimulation of serotonin receptor sites. As a first step in delineating the mechanisms accounting for the status-linked difference in response to serotonergic drugs, we hypothesized a difference at the level of the 5-HT2 receptor sites between brain regions of dominant and subordinate vervet monkeys. An additional hypothesis, that the difference lies in membrane responsiveness or interactions with other neurotransmitters, is possible. This report describes the specific binding of the 5-HT2 ligand ketanserin and its displacement by quipazine in vervet monkey brain tissue.

TABLE 1

SPECIFIC KETANSERIN IN BINDING IN AREAS OF BRAIN FROM DOMINANT AND SUBORDINATE VERVET MONKEYS*

Brain Area	Dominant	Subordinate
Orbitofrontal Cortex	140 ± 6	150 ± 11
Posterior Orbitofrontal Cortex	125 ± 14	139 ± 17
Medial Frontal Cortex	138 ± 10	118 ± 15
Superior Frontal Cortex	114 ± 16	139 ± 12
Inferior Frontal Cortex	116 ± 12	117 ± 20
Temporal Tip Cortex	114 ± 13	114 ± 15
Medial/Inferior Parietal Cortex	104 ± 09	106 ± 04
Occipital Cortex	86 ± 13	104 ± 09
Uncus/Amygdala	63 ± 11	48 ± 14
Hippocampus	39 ± 08	52 ± 08
Hypothalamus	39 ± 06	32 ± 04
Thalamus	15 ± 04	23 ± 03
Midbrain	12 ± 02	14 ± 03

*Specific binding (mean ± SEM) in femtomoles/mg protein from eight dominant/subordinate pairs is represented. Specific ketanserin (3 nM) was displaced with 3 μM cyproheptadine.

METHOD

Adult, male vervet monkeys *(Cercopithecus aethiops sabaeus)* were used as subjects in this study. All animals were feral caught on the islands of St. Kitts or Nevis, Eastern Caribbean, and were housed in social groups either on St. Kitts or at the Nonhuman Primate Laboratory, Sepulveda Veterans Administration Medical Center, Sepulveda, CA. The social groups consisted of 3 adult males, 2–4 adult females, and immature offspring. Characteristics of enclosures and care were as previously described [10]. The adult composition of each social group had been constant for at least 6 months prior to these investigations. The dominant and subordinate males in each group were identified by their percentages of success in dyadic, intermale agonistic encounters. The identity of the dominant male in each group was consistent for at least 2 months prior to the onset of the studies and was independently determined by at least 2 observers.

The dominant male and a randomly selected subordinate male in each group were sacrificed. Animals were sacrificed between 07.00–12.00. The dominant/subordinate pair from each group were harvested the same day, and the selection of the dominant animal as the first or second animal taken was alternated between groups.

Tranquilization of subjects was obtained with ketamine (8–14 mg/kg, IM). After the collection of final peripheral samples, deep anesthesia was obtained using pentobarbital. Administration was conducted IV in small increments until the loss of the corneal eyeblink reflex and the relaxation of skeletal musculature. Further pentobarbital treatment was avoided to spare respiration, and no animal was responsive to any of the remaining procedures. Breathing was shallow, but was regular and was maintained unaided. The brain was removed intact and chilled in iced saline for about 20 min prior to dissection on ice. Cortical and subcortical brain areas were isolated by free-hand dissection and frozen in either dry ice or liquid nitrogen.

The location of the sampled areas of cortical tissue are indicated in Fig. 1. Pia mater was stripped from the areas to be sampled. The cortical areas were blocked with scalpel or scissors cuts and removed. Deep white matter was removed, but a patchy, shallow layer (<0.5 mm) was allowed to remain so as not to jeopardize the deepest cortical layers. The medial surface and the orbital and rostral sulci were used to demark the orbitofrontal cortex, with the division between orbitofrontal and posterior orbitofrontal areas being midway between the optic chiasm and the frontal pole. The posterior orbitofrontal cortical area was further bounded by the olfactory tract and the frontomarginal sulcus, extended. The posterior orbitofrontal area was unusually shallow for cortex, being apposed by the caudate. The occipital cortical sample was deep and included the line of Gennari. Anterior pieces of hippocampus (including dentate) were removed from the temporal tip and uncus (including amygdala). The inferior surface of the temporal lobe was opened, the hippocampus/dentate exposed by blunt dissection, and an approximately 15 mm length (including the most anterior fragments) sampled. The head and body of the caudate were exposed, and the caudate, putamen, and globus pallidus, as well as cortical remnants, were blunt dissected away along the lines of the internal capsule. The hypothalamus was blocked with reference to the anterior commisure, the fornix, the mammillary bodies, and the reflected optic tract. The midbrain sample included the colliculi dorsally and was bounded ventrally by the mammillary bodies and the transverse band of the pons.

The preparation of brain tissue for ligand binding was similar to that reported by Schotte *et al.* [13] for human brain samples. In particular, thawed samples were initially homogenized in 20 volumes of a Tris buffer (50 mM Tris, 5 mM $MgSO_4$ 0.5 mM EGTA, pH 7.4 at room temperature) and centrifuged (10,000 g × 10 min). The pellet was rehomogenized and pelleted once again before final resuspension in Tris buffer to a final tissue concentration of about 25 mg (wet weight) per ml. Assays were conducted in a total volume of 600 μl. [3H]-Ketanserin of high specific activity (≥75 Ci/mmole) was obtained commercially (New England Nuclear). Specific binding was determined from the difference in radioactivity between total ligand bound and ligand bound in the presence of a competing agent. Five tubes (3 total and 2 non-displaced binding) were used to determine each specific binding point. Three types of ligand binding studies were conducted. (1) Specific binding was determined at nine ketanserin concentrations ranging 0.1–3 nM for the determination of Kd and Bmax. (2) Specific binding was determined at 3 nM ketanserin to compare different brain regions. (3) The inhibition of ketanserin binding at 2 nM by quipazine concentrations ranging 0.1–10 μM to calculate IC50 for quipazine. In all cases, non-specific ketanserin bound was determined in the presence of 3 μM cyproheptadine. At 3 μM, cyproheptadine was 1000-fold in excess over the highest ketanserin concentration employed and was >1000-fold in excess over the Ki for cyproheptadine inhibition of ketanserin binding in rat cortex [6]. The binding period was initiated by the addition of 500 μl tissue preparation, and the tubes were incubated 15 min at 37°C. Free and bound ligand were separated by the rapid addition of 3 ml iced Tris buffer per tube followed immediately by filtration over glass fiber filters (S&S No. 32) and immediate rinsing of the filter (2×3 ml). Air was drawn through the filters for 5–10 min, and radioactivity was measured by scintillation counting. Protein content of the tissue preparation was determined by the Lowry method after alkaline digestion of particulates [7].

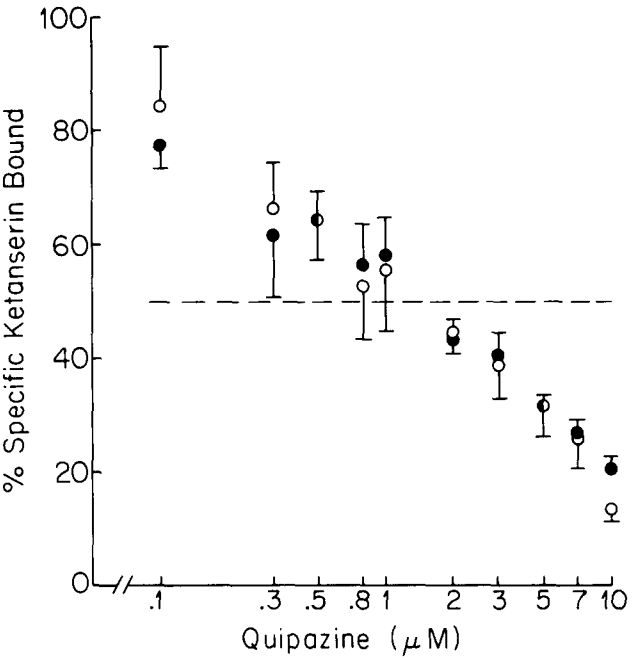

FIG. 2. Quipazine inhibition of specific ketanserin binding. The displacement of specifically bound ketanserin (2nM) by a range of quipazine concentrations was examined. The "zero" percent specifically bound remaining level was that remaining in the presence of 3 μM cyproheptadine. Mean values combined from analysis of premotor and occipital cortex of 3 dominant/subordinate pairs are plotted. Vertical lines represent standard errors of the means. ●=dominant animals. ○=subordinate animals. Regression analysis yields 50% inhibition at 1.4 μM in cortical tissue from dominant animals and at 1.3 μM in tissue from subordinate animals.

Whole blood serotonin was assayed by the fluorescence method of Yuwiler *et al.* [14] as described previously [10].

RESULTS

The specific binding in membranes of cortical areas selected from the frontal, temporal, and occipital cortex of 3 dominant/subordinate pairs was examined at nine ketanserin concentrations ranging from 0.1–3 nM. Linear regression analysis of Eadie-Hofstee plots was used to calculate Bmax and Kd. No status-related differences were observed in the density of ketanserin sites, Bmax, nor in Kd, the affinity constant. Because not all cortical areas were examined in all animals and the binding levels were different between cortical areas, a correlated *t*-test comparing each area per dominant/subordinate pair was employed. Mean cortical Bmax was 130±10 (Mean±SEM, N=5) femtomoles/mg protein for samples from dominant animals and was 132±15 for samples from subordinate animals. Similarly, Kd calculated from measurements on tissue from dominant animals was 0.39±0.05 nM and from subordinate animals was 0.48±0.12. Specific binding (femtomoles/mg protein) measured at a single ligand concentration of 3 nM was independent of tissue level over the range employed in these studies (0.75–2.5 mg protein/ml). Specific binding was independent of incubation time between 10 and 30 min. Over the range of ketanserin concentrations examined, specific bound DPM was 67%±2 (Mean±SEM) of total DPM bound.

The specific binding of ketanserin at 3 nM was determined in 13 cortical and subcortical brain areas from 8 pairs of dominant and subordinate vervet males. The results are recorded in Table 1. No differences in the mean specific ketanserin bound were observed between dominant and subordinate groups. WBS measured at the time of sacrifice was, for dominant males, 1080±146 ng/ml (Mean±SEM), and 733±50 for subordinate animals (correlated $t=3.00$, $p=0.024$). In addition to there being no difference between mean ketanserin binding for dominant and subordinate groups, examination of each dominant/subordinate pair indicated no suggestion of any trend toward dominant/subordinate difference. A modest anterior-posterior gradient in ketanserin binding among cortical sites and the relative paucity of binding in subcortical areas was apparent.

The displacement of ketanserin at 2 nM by a concentration range of quipazine is depicted in Fig. 2. Data from premotor and occipital cortex from 3 dominant/subordinate pairs were combined in this figure. No difference in the quipazine displacement of specific ketanserin binding was observed between dominant and subordinate animals. Regression analysis indicated IC50 of 1.4 μM and 1.3 μM in membranes from dominant and subordinate animals, respectively.

DISCUSSION

We have examined brain areas of dominant and subordinate vervet monkeys and have found no status-related differences in specific ketanserin binding, nor in the derived density and affinity parameters, Bmax and Kd. Differences were hypothesized because of the common behavioral effects of tryptophan, fluoxetine, and quipazine. These results suggest that dominant and subordinate vervet males do not differ in the number of CNS 5-HT2 receptors.

The regional differences observed in ketanserin binding were similar to reports of other investigators. Divac *et al.* [3] using spiroperidol (mianserine displacement) binding in vervet cortical areas similarly found that orbital regions exhibited the highest specific binding, the occipital pole somewhat lower binding, and the amygdala the lowest binding of all (other subcortical tissues were not reported). Schotte *et al.* [13] reported the regional distribution of ketanserin binding (methysergide displacement) in human brain. As with the vervet, human orbital gyrus exhibited the second highest binding level reported, and the binding at the occipital pole was about $^2/_3$ that in frontal areas. Among subcortical areas, Schotte *et al.* [13] reported binding in hippocampus and thalamus to be, respectively, about $^1/_2$ and $^1/_4$ the level observed in the occipital cortex.

Quipazine displacement of ketanserin binding provided a second approach to 5-HT2 receptors. Quipazine use was based on two important features, its ability to elicit status-related behavioral changes and the fact that quipazine's behavioral effects seem largely to be exerted via 5-HT2 receptors [4]. Because of its low affinity for the ketanserin binding site [6], quipazine would dissociate too fast to serve as the labeled ligand in the direct examination of these sites. However, quipazine displaces ketanserin [6], and this displacement was compared between tissue from dominant and subordinate animals. That no status-related difference in quipazine displacement of ketanserin binding was observed further suggests that dominant and subordinate vervet males do not differ at the level of CNS 5-HT2 receptors and that other or additional mechanisms account for the status-related drug response differences.

The selection of brain areas for examination was based upon ablation studies in primates [5,9] and studies in reptiles [1] identifying areas involved in some aspect of dominant behavior. Clearly, we may have missed a critical area, or a small critical area may have been obscured in the larger tissue samples examined. That some 5-HT2 receptors are involved in the status-linked responses following tryptophan, fluoxetine, and quipazine treatment is suggested by the fact that while these agents all increase the occurrences of "Groom" and "Approach" and decrease "Locomote" and "Be Solitary," cyproheptadine, a classical serotonin antagonist affecting 5-HT2 sites, elicits opposite changes in these behaviors (M. J. Raleigh, unpublished).

In addition, other 5-HT receptor sites such as the serotonin uptake recognition site, its associated, imipramine-sensitive regulator site, the auto-receptor site, or the more recently identified subtypes of 5-HT1 receptors may be involved. Finally, status-related differences in receptor-coupled membrane polarization changes or interactions with other neurotransmitter systems are possible.

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