Correction. In the article "Human serum Cohn fraction IV (α-globin enriched) inhibits ligand binding at neurotransmitter receptors in human brain" by Anne C. Andorn, Miguel A. Pappolla, Hans Fox (Fuchs), Frank K. Klemens, and Pamela A. Martello, which appeared in number 12, June 1986, of Proc. Natl. Acad. Sci. USA (83, 4572-4575), the title is incorrect because of a printer's error. The correct title is "Human serum Cohn fraction IV (α-globulin enriched) inhibits ligand binding at neurotransmitter receptors in human brain."
Human serum Cohn fraction IV (α-globin enriched) inhibits ligand binding at neurotransmitter receptors in human brain

(\([\text{\textsuperscript{3}H}\text{spiroperidol binding/serotonin receptors/dopamine receptors}]\))

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Communicated by Oscar D. Rainoff, February 28, 1986

ABSTRACT Human serum proteins are found in significant density in the neuropeptides in brains of demented individuals. The functional significance of these abnormally distributed proteins has been unknown. We now report that α-globulin-enriched fractions of human serum decrease the specific binding of [\text{\textsuperscript{3}H}\text{spiroperidol} at its binding sites in postmortem human frontal cortex and caudate. The substances in this serum fraction apparently exert their effect by a direct action on the binding site. Since [\text{\textsuperscript{3}H}\text{spiroperidol} labels serotoninergic and dopaminergic among other neurotransmitter receptors, these results suggest that components of human serum inhibit the binding of ligands at neurotransmitter receptors.

Serum proteins are found in abnormal locations within the brain in many neuropsychiatric diseases, including presenile and senile dementia (1–3, 32) and to a lesser extent in “normal” aging (unpublished observations). The functional significance and physiological consequence of these abnormally placed serum proteins have not been known (1–3). Brain neurotransmitter receptor density can be decreased in aging and is often significantly reduced in some diseases of the nervous system (4–14). In particular, brain serotonin receptor density is reduced in aged brain cortex but is even more markedly reduced in dementia of the Alzheimer type (15). The antipsychotic ligand [\text{\textsuperscript{3}H}\text{spiroperidol} binds at serotonergic-like binding sites in human frontal cortex and at dopamine receptors in human caudate (16–18). Human serum has been shown to decrease the binding of this ligand by 20–30% at its binding sites in rat striatum (16). We hypothesized that the functional consequence of abnormally located serum proteins might be the inhibition of ligand binding at neurotransmitter receptors. The following studies were performed to test this hypothesis and the results indicate that an α-globulin-enriched human serum fraction decreases ligand binding at neurotransmitter receptors by a direct action on the binding site.

MATERIALS AND METHODS

Tissue Retrieval and Preparation. Human brains were obtained at the time of autopsy in strict accordance with the guidelines of the institution. Only normal brains were used for this study and were defined as those obtained from individuals dying of sudden accidental or cardiovascular death with no antecedent or concurrent neurological or psychiatric illness and no pathological findings unrelated to the cause of death. A total of 25 postmortem specimens was used for these studies, with a mean (± SD) age of 37.5 ± 11.0 years (range, 18–53 years) and a mean (± SD) postmortem interval of 19.1 ± 14.9 hr (range, 7–72 hr).

Mouse 

Tissue Preparation. The frontal lobe (preorbital area, including Brodmann areas 9–12) was dissected at autopsy and the meninges were removed. The cortex was removed by manual scraping. The caudate was removed bilaterally. Each tissue was minced and placed in vials containing ice-cold buffer consisting of 20 mM NaHepes (pH 7.4 at 4°C) and 2 mM MgSO\(_4\). This tissue was then quickly frozen in a dry ice/acetone bath and stored at −70°C as described (17). At the time of preparation the tissue was quickly thawed at 37°C and diluted 1:10 (vol/vol) in 20 mM NaHepes (pH 7.4 at 4°C) with 2 mM MgSO\(_4\) and homogenized by 10 strokes in a hand-held glass/Teflon homogenizer on ice. The homogenate consisted of broken cells, organelles, and swollen nuclei by light microscopy (not shown). This homogenate was brought to a final 1:50 dilution of the original minced tissue (vol/vol) in the same buffer. This suspension was centrifuged at 39,000 x g at 4°C for 20 min. The supernatant was discarded and the pellets were rinsed twice with 5 ml of fresh buffer without disruption. The pellets were then resuspended in fresh buffer to the 1:50 dilution of the original mince and the washing procedure was repeated three more times in fresh buffer. The final rinsed pellets were suspended in the same buffer (pH 7.4 at 37°C) at a concentration of 20–40 mg of protein per ml of suspension. This final suspension consisted primarily of particulate membrane fragments of central nervous system origin, deriving from neuronal, glial, microvascular and intracellular membranous structures, and nuclei. Protein concentrations were determined by the method of Lowry et al. (19) with bovine serum albumin in the NaHepes/MgSO\(_4\) buffer as a standard. Aliquots of the final particulate fractions were quickly frozen and stored as above. Prior to use in a binding assay, prepared particulate fractions were quickly thawed at 37°C and brought to a final concentration of 2–4 mg of protein per ml in the same buffer, pH 7.4 at the temperature of the incubation.

Binding Assays. The radioligand binding studies used were as described (17). [\text{\textsuperscript{3}H}\text{spiroperidol} was dried under N\(_2\) and quickly resuspended at 4°C) in 200 mM NaHepes (pH 7.4 at temperature of incubation), 20 mM MgSO\(_4\), and 1.0 mM ascorbate. This suspension and other ingredients were added to glass test tubes for a final concentration of reagents as follows: 0.2 nM [\text{\textsuperscript{3}H}\text{spiroperidol}, 0.1 mM ascorbate, 34.4 mM NaHepes, 3.4 mM MgSO\(_4\), additions as stated, and 0.2 ± 0.05 (mean ± SD) mg of particulate protein. The reaction mixture was allowed to incubate for 60 min at 21°C (frontal cortex) or for 60 min at 37°C (caudate). The 21°C temperature was chosen for frontal cortex because at 37°C there is a time-related loss of specific [\text{\textsuperscript{3}H}\text{spiroperidol} binding that

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resembles receptor down-regulation in this tissue (17, 33). Physiologic temperature (37°C) could be used for the caudate because specific [3H]spiroperidol binding in this tissue maintains a steady state at 37°C (18). The binding reaction was terminated by rapid filtration over Schleicher & Schuell no. 30 glass fiber filters that had been presoaked in wash solution (see below) and rested on ice-cold stainless steel filter heads (Hoefer, San Francisco). The filters were washed with 15 ml of wash solution consisting of 5 mM NaHepes (pH 7.4 at 4°C) and 2 mM MgSO4. The filters were then dried under full vacuum. The entire filtration and washing procedure was completed in <6 sec. The dried filters were placed in scintillation vials to which 4 ml of Beckman Econosolv was added. Radioactivity was determined by liquid scintillation spectrophotometry (Beckman LS 7500) at an efficiency of 30%. Specific binding was determined as the difference between the amount of [3H]spiroperidol binding occurring in the absence (total binding) and in the presence (nonspecific binding) of 10 μM haloperidol. Total binding and nonspecific binding were determined in the presence of each concentration of human serum protein component studied.

In some experiments, prepared particulate membrane fractions were exposed to various serum protein fractions prior to use in a binding assay. This was done by diluting prepared particulate fractions to 0.2 ± 0.05 mg of protein per ml in 20 mM NaHepes (pH 7.4 at the temperature of preincubation) and 2 mM MgSO4. The additions were then made and the suspension was preincubated at the temperatures and for the times stated below. For preincubation studies, the suspension was then centrifuged at 39,000 × g for 20 min, and the pellets were rinsed, resuspended, and used directly in a binding assay (at 0.2 mg of particulate protein per ml assay). For preincubation and washing studies, the pellets from the step above were resuspended in 50 vol of buffer (vol/vol) and washed four times in fresh buffer, as per our standard preparation procedure, prior to final resuspension and use in the binding assay at 0.2 mg of particulate protein per ml of assay.

Reagents. [3H]spiroperidol was obtained from Amersham at a specific activity 16–20 Ci/mmol (1 Ci = 37 GBq) and was stored at −20°C. Thin-layer chromatography in multiple solvent systems was used to demonstrate >99% ligand purity prior to use and during continued use of the compound. Human serum Cohn fraction IV (α-globulin enriched), human serum Cohn fraction II (γ-globulin enriched), and human IgG were obtained from Sigma and Miles. Bovine serum Cohn fraction II and human albumin were obtained from Miles. Haloperidol was the generous gift of Janssen Pharmaceuticals (New Brunswick, NJ). All other chemicals were obtained from Sigma.

Accuracy and Reproducibility. Sufficient cpm were obtained to effect a counting error of <5%. All experiments were replicated on different postmortem specimens and all experimental points were obtained in at least duplicate, with <7% coefficient of variation between duplicates. Results are reported as the mean ± SD, or as stated.

RESULTS
Human serum Cohn fraction IV obtained from the sources above was 60–70% α-globulin as determined by electrophoresis in our laboratory (not shown). Human serum Cohn fraction IV decreased specific [3H]spiroperidol binding in human frontal cortical particulate fractions in a dose-dependent fashion, with an IC50 of 1.1 ± 0.1 mg of Cohn fraction IV per ml of assay (n = 6) in five different postmortem specimens (Fig. 1). This human serum fraction also decreased specific [3H]spiroperidol binding in a dose-dependent fashion in human caudate (Fig. 1) and the IC50 for this effect was 1.4 ± 0.8 mg of Cohn fraction IV per ml of assay (n = 5) on four different postmortem specimens.

To determine whether this effect was due to a direct action of human serum Cohn fraction IV on the [3H]-labeled ligand binding sites, frontal cortical particulate fractions from each of two postmortem specimens were prepared. Aliquots of each preparation were untreated or preincubated in human serum Cohn fraction IV and used directly or extensively washed prior to use. As shown in Fig. 2, specific [3H]spiroperidol binding was significantly reduced in frontal cortical particulate fractions preincubated in Cohn fraction IV regardless of washing.

To ensure that the marked decrease in specific [3H]spiroperidol binding was due to action of Cohn fraction IV and not to preincubation alone, or preincubation, centrifugation, and resuspension of the tissue, the same experiments were performed without the addition of human serum Cohn fraction IV during the preincubation step. Duplicate experiments on two postmortem specimens showed that the amount of specific [3H]spiroperidol binding in the fractions preincubated in buffer alone was 104.6% ± 0% of the amount in the untreated fractions. In experiments on three postmortem specimens, companion particulate fractions were either untreated or preincubated in buffer alone and washed. The amount of specific [3H]spiroperidol binding in the preincubated and washed fractions was 85.0% ± 13.5% of that in the untreated fractions.

Dose–response studies were also performed employing various human serum and bovine serum proteins. Bovine serum Cohn fraction II at 10 mg/ml of assay decreased specific [3H]spiroperidol binding in frontal cortex by 39.7% ± 8.4% (n = 3), whereas human albumin at 10 mg/ml of assay decreased specific [3H]spiroperidol binding by 26.3% ± 6.0% (n = 3). Human IgG at the same concentration also produced only a 28.9% ± 9.8% decrease in binding. These findings are in contrast to those observed with Cohn fraction IV, which at 10 mg/ml of assay consistently decreased [3H]spiroperidol binding.
binding by >90%. Human serum Cohn fraction II (gamma globulin enriched) decreased specific \[^{3}H\]spiroperidol binding in frontal cortex and caudate in a dose-dependent fashion, with IC\(_{50}\) values of 3.4 ± 0.8 (n = 12) and 2.8 ± 1.1 mg of Cohn fraction II per ml of assay (n = 5), respectively. However, the same samples of Cohn fraction II that produced these results did not decrease \[^{3}H\]spiroperidol binding even at concentrations as high as 60 mg/ml of assay in other experiments on the same postmortem specimens. In fact, a total of 36 dose-response studies was performed under a variety of conditions in frontal cortex and caudate. However, although 21 of these showed gamma globulin-enriched fractions to be effective, under the same conditions, 12 showed gamma globulin to be ineffective. In contrast, the results obtained with Cohn fraction IV were consistently reproducible.

To determine whether human albumin decreased \[^{3}H\]spiroperidol binding by a direct action on the binding site, frontal cortical particulate fractions were preincubated for 60 min at 21°C in human albumin at a ratio of 50 mg of human albumin per 0.2 mg of particulate protein (five times the concentration of human a-globulin-enriched fractions used above). Compared to control conditions, only 61.5% ± 8.6% of specific \[^{3}H\]spiroperidol binding was present in tissue preincubated in human albumin. Nearly all of this binding was recovered with washing, since 92.2% ± 8.9% of control specific \[^{3}H\]spiroperidol binding was present in the tissue preincubated in human albumin and then extensively washed (n = 3).

**DISCUSSION**

\(\alpha\)-Globulin-enriched human serum fractions decreased the specific binding of \[^{3}H\]spiroperidol at its binding sites in human frontal cortex and in caudate in a dose-dependent fashion (Fig. 1). The IC\(_{50}\) for this effect in either tissue is below normal serum concentrations of a-globulin, which range from 5 to 16 mg/ml of serum (20).

The major decrement in specific \[^{3}H\]spiroperidol binding induced by \(\alpha\)-globulin-enriched human serum fractions is apparently due to a direct effect of constituents of this fraction on the binding site and not to \(^{3}\)H-labeled ligand-serum protein interactions, as suggested (16). If frontal cortical particulate fractions are exposed to human serum Cohn fraction IV and then extensively washed, removing free serum proteins prior to use in a binding assay, the decrement in specific \[^{3}H\]spiroperidol binding is nearly the same as in untreated particulate fractions assayed in the presence of human serum Cohn fraction IV (Fig. 2). Further, there is little apparent recovery of specific \[^{3}H\]spiroperidol binding in comparing tissue preincubated in \(\alpha\)-globulin with preincubated and extensively washed tissue. Since the particulate membrane fractions derive from neuronal, glial, and microvascular sources, we cannot be certain of the location of these binding sites. However, in two frontal cortical preparations from which microvessels and nuclei had been removed by three 1000 × g centrifugations of the homogenate, \(\alpha\)-globulin still decreased \[^{3}H\]spiroperidol binding, with IC\(_{50}\) values of 1.3 and 2.3 mg/ml of assay. Therefore, some of the effects of a-globulin are on binding sites derived from neuronal and/or glial membranes.

The decrease of specific \[^{3}H\]spiroperidol binding observed in particulate fractions preincubated in \(\alpha\)-globulin-enriched fractions is not due to the effects of preincubation in buffer or subsequent centrifugation and resuspension of the particulate fractions. Preincubation of the particulate fractions in buffer alone had no effect on specific \[^{3}H\]spiroperidol binding. Preincubation of the particulate fractions followed by washing and resuspension produced a <15% decrease in specific \[^{3}H\]spiroperidol binding. But, preincubation of the particulate fractions in \(\alpha\)-globulin-enriched serum fractions followed by washing of the fractions produced a 76.0–79.3% decrease of specific \[^{3}H\]spiroperidol binding (n = 2).

Some other serum proteins did not apparently produce the same effect as human serum Cohn fraction IV. Human albumin did decrease \[^{3}H\]spiroperidol binding in human frontal cortex by an apparently weak but direct action on the binding site, but this effect was readily reversible, unlike the inhibiting effect of a-globulin-enriched fractions. Due to its inconsistency, we cannot be certain that gamma globulin-enriched fractions did indeed produce the same effect as \(\alpha\)-globulin-enriched fractions.

We cannot tell from these data whether the apparent binding of constituents of human serum Cohn fraction IV to antipsychotic drug receptors represents an effect of a-globulin alone. Although all lots of the human serum Cohn fraction IV (n = 4) used in these studies were at least 60–70% \(\alpha\)-globulin, it was certainly not the only potential reactant. Further, although all lots of human serum Cohn fraction II (n = 7) used for these studies were >98% gamma globulin, the inconsistency of the results obtained suggest that the reactant may be a protein associated to the globulins.

The intracerebral presence of serum proteins may indicate a nonspecific macromolecular leak through the blood–brain barrier (21–23). It is assumed that under normal conditions appreciable quantities of serum proteins do not pass the endothelial cell layer of the brain microvasculature (21–23).
Among the variety of experimental and clinical situations in which blood–brain barrier defects have been demonstrated are presenile and senile dementia, acute and chronic hypertension, ischemia, seizures, infections, increased serum serotonin concentrations, spontaneous brain tumors, and brain trauma (21). In many of these cases it has been demonstrated that globulins are abnormally present in the neuropil, in neurons, and as a component of senile cerebral amyloid (1–3).

Most recently, abnormal myeloma proteins (immunglobulins) have been demonstrated intraneuronally in multiple myeloma (24). We have also demonstrated the presence of α-globulin, gamma globulin, and IgM intraneuronally in Parkinson disease (25). We have demonstrated here that human α-globulin-enriched fractions decrease specific antipsychotic ligand binding in human frontal cortex and in caudate. They apparently do so by a direct and ligand-independent action on the binding sites.

In human frontal cortex, [3H]spiroperidol binds to serotonergic-like sites (17), which most likely represent the serotonin 2 receptor and perhaps the serotonin 1A receptor as identified in the lower mammalian cortex (26–28). Serotonin receptors are known to be decreased in aging (13, 15). In human caudate, [3H]spiroperidol binds at dopaminergic-like binding sites (18), most likely the dopamine 2 receptor as demonstrated in the lower mammalian striatum (6, 7, 16, 29). These receptors are also known to be decreased in aged human basal ganglia (11). The intra- and interneuronal presence of serum proteins seen in aged and demented brains may therefore have important functional consequences by decreasing ligand binding at neurotransmitter receptors. Furthermore, perhaps the reduction of receptor density seen in aging reflects the presence of these proteins.

Alternatively, serum protein blockade of neurotransmitter receptors could provide a homeostatic mechanism for the preservation of the integrity of the blood–brain barrier. Pharmacologically active serotonin 2 receptors are postulated to be present on the luminal surface of central nervous system microvasculature. Serotonin acts through serotonin 2 receptors to increase vascular permeability (30). One might speculate that serum proteins help maintain the impermeability of the microvasculature by blocking the action of serotonin at its receptors. However, only minimal specific [3H]spiroperidol binding has been demonstrated in lower mammalian central nervous system microvasculature (31). So this possible mechanism may be highly unlikely. Nonetheless, serum protein–neurotransmitter receptor interactions warrant further investigation and consideration in the etiology of the above disease states and of human aging.

We especially thank Mrs. Judith Stitts and Ms. Holly Perzy for expert technical assistance. We are also grateful for the support of the Genetics Center, Case Western Reserve University, and the Department of Chemistry, John Carroll University. This work was supported by National Institute of Neurological and Communicative Disorders and Stroke Teacher–Investigator Award NS04648 and National Institutes of Health Grant AG00460 to A.C.A. and a Case Western Reserve University School of Medicine Summer Fellowship to H.F.