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Human Brain Receptor Alterations in Suicide Victims

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MEYERSON, L. R., L. P. WENNOGLE, M. S. ABEL, J. COUPET, A. S. LIPPA, C. E. RAUH AND B. BEER. *Human brain receptor alterations in suicide victims.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 159–163, 1982.—A comparison was made of human postmortem muscarinic-cholinergic, β-adrenergic and serotonergic (presynaptic) recognition sites in cortical tissues derived from suicide and homicide (control) victims. An elevation of 47% and 35% in the suicide group compared to controls was observed in receptor ligand binding for ^{3}H-quinuclidinyl benzilate (QNB, muscarinic antagonist) and ^{3}H-imipramine (IMI, a presynaptic serotonin marker), respectively. In contrast, no appreciable differences in ^{3}H-dihydroalprenolol (DHA, β-adrenergic antagonist) binding were observed between the two groups. Additionally, tissues from both groups of subjects were analyzed for tricyclic antidepressive agent (TAD) content. High performance liquid chromatographic (HPLC) tissue analysis revealed no detectable levels of tricyclic agents with an assay sensitivity of 50 picograms/mg tissue. The results presented herein demonstrate neurotransmitter-receptor alterations in suicide subjects compared to homicide (control) victims. The attendant roles of serotonergic and muscarinic-cholinergic processes in the psychobiology of suicide and depression are addressed.

Depression Imipramine Cholinergic receptors Suicide Serotonin

SUICIDE is a phenomenon which is the target of extensive research. While the prime focal points revolve around demographic, social and psychological variables, accounts of biological-related information are particularly lacking. An understanding of the relationship between depression and suicide is unclear. Although the incidence of clinical depression in suicides is well documented, a statistical collation of such studies revealed that 12–64% of suicide victims were previously diagnosed as clinically depressed [24]. Therefore, not all suicides on record have been directly related to depressive illness, and may involve unique etiologies.

Several lines of evidence point to the interactions of aminergic systems in the factors underlying suicide. For example, Bourne, *et al.* [4] found significantly lower levels of 5-hydroxyindoleacetic acid (5-HIAA) in hindbrains of suicide victims compared to controls, whereas no alterations in endogenous serotonin or norepinephrine levels were observed. Furthermore, the incidence of diminished 5-HIAA concentrations in the cerebrospinal fluid of persons attempting suicide has been observed [3]. Another biological variable consistently related to suicide is low monoamine oxidase activity in platelets [9].

The role of the cholinergic system with respect to its involvement in suicide and depression is even more obscure. For instance, administration of either acetylcholine precursors such as choline or the organophosphate anticholinesterases induce clinical symptomatologies of depression [5,21]. In addition, the anticholinergic agent biperiden was demonstrated to produce significant improvement in severely depressed patients [13]. Janowsky and co-workers [12] advanced the "cholinergic-adrenergic hypothesis of depression" which implicates an imbalance caused primarily by a hyperexcitability of cholinergic neurons, leading to a secondary inhibition of adrenergic neurons.

Thus, with these constructs at hand, it was pertinent to extend the observations in suicide populations to assess their attendant neuronal recognition sites for alterations in the cholinergic-muscarinic (^{3}H-QNB), serotonergic (^{3}H-IMI) and β-adrenergic (^{3}H-DHA) systems. To this endpoint a profile of cortical receptor binding site characteristics was examined in suicide and homicide victims. To ensure that any potential differences in receptor characteristics were not due to prior treatment with tricyclic antidepressive agents (TADs), analytical studies quantifying the presence of TADs in tissues studied were conducted.

METHOD

Tissue Acquisition

Postmortem human brain tissues were obtained from Dr. M. Stanley of the Manhattan VA Medical Center through the Medical Examiners Office of New York City. Control human brain tissues were those from individuals who died suddenly of non-neurological causes (i.e., auto accidents, n=7 or homicides involving weapons, n=3). Brain tissues were also obtained from suicide victims (i.e., death via hanging, n=4 or gun, n=4). Data were collected with respect to subject age, cause of death, height, weight, gender, race and interval between death, tissue excision (<48 hours) and freezing (−20°C). Samples were stored frozen for 9 months prior to

analysis. Information as to prior drug therapy in the subject population was not available. Tissue specimens were stated to be frontal cortical blocks, however, inclusion of varying degrees of adjacent brain structures cannot be fully excluded.

Tissue Preparation

Frozen cortical tissue (−20°C) was weighed, thawed and homogenized (20:1 V/W) in 50 M Tris HCl, pH 7.4, using a Tekmar tissumizer (50% power, 15 sec). An aliquot of the homogenate was removed for ^{3}H-QNB binding experiments. The remaining tissue homogenate was centrifuged at 49,000 ×g for 15 minutes. The resultant pellet was suspended in 50 mM Tris HCl, pH 8.0 and an aliquot was removed and frozen for subsequent ^{3}H-DHA binding experiments. The remaining suspension was recentrifuged and the pellet was suspended in 50 mM Tris buffer (pH 7.5) containing 0.12 M NaCl, 0.005 M KCl, and frozen until use for ^{3}H-IMI binding experiments.

Receptor Binding Analysis

^{3}H-QNB binding. ^{3}H-QNB binding parameters were determined as described elsewhere [25]. Cortical membranes (100 μg protein) were incubated at 25°C for 60 minutes in 50 mM Tris HCl, pH 7.4, containing ^{3}H-L-QNB (40.2 Ci/mmol, New England Nuclear Corp.) Saturation isotherms were determined using a ligand concentration range of 0.005–0.2 nM. Nonspecific binding was determined using 100 μM oxotremorine. Each assay was performed in triplicate in a total volume of 2 ml. The reactions were terminated by the addition of 5 ml cold buffer and twice washed by rapid vacuum filtration through Whatman GF/C filters. Radioactivity was determined with a Beckman LS-7500 liquid scintillation spectrometer in all binding assays.

Data from saturation isotherms were analyzed by the method of Scatchard [18] and best fitting lines were determined by least squares linear regression analysis.

^{3}H-DHA binding. Cortical membranes were assayed for specific ^{3}H-DHA binding using a modification of methods described elsewhere [1,10]. Briefly, membranes (300 μg protein) were incubated at 23°C for 15 minutes in 50 mM Tris HCl, pH 8.0, containing ^{3}H-DHA (101 Ci/mmol, New England Nuclear Corp). Because of a paucity of tissue, specific binding was determined at two ligand concentrations, 0.5 and 1.0 nM. Nonspecific binding was determined for each concentration by including 1.0 μM propranolol in the assay mixture. Each assay was performed in quadruplicate in a total volume of 250 μl. The reactions were terminated by the addition of 5 ml of ice cold buffer and the filters were twice washed by rapid filtration under vacuum through Whatman GF/B filters.

^{3}H-IMI binding. Cortical membranes were assayed for ^{3}H-IMI binding using established procedures [15,23]. Briefly, membranes (400 μg protein) were incubated at 0°C for 90 minutes, with ^{3}H-IMI (31.9 Ci/mmol, New England Nuclear Corp), at two ligand concentrations, 2.2 and 5.3 nM. Nonspecific binding was determined for each concentration by including 10 μM desipramine in the reaction mixture. Each assay was performed in triplicate in a total volume of 250 μl. The reactions were terminated by the addition of 5 ml ice cold buffer and the filters were twice washed by rapid filtration under vacuum through Whatman GF/B filters.

Tissue Analysis of Tricyclic Antidepressive Agents

An ion-pair reverse phase HPLC method using both electrochemical and ultraviolet (280 nm) detection was employed for the quantification of existent TAD concentrations in cortical tissue from both suicide and homicide victims [20]. This method is capable of quantitatively detecting imipramine, desipramine, amitriptyline, and their respective hydroxymetabolites. Together these represent greater than 90% of the available tricyclics prescribed in the USA. Briefly, 1 ml of individual brain homogenates containing 200 mg tissue were extracted at pH 9.7 with diethylether, back extracted into 1.0 N HCl and re-extracted into ether after alkalinization. The dual electrochemical and UV detection system affords a sensitivity detection level as low as 50 picograms/mg tissue in a mobile phase of acetonitrile-acetate buffer (40:60) containing 0.005 M heptanesulfonate. Calibration curves were achieved by extraction of control tissue spiked with varying concentrations of authentic drug standards mentioned above.

Statistical Analysis

Two methods of statistical analysis were employed. First, means of each group were compared using Student's *t*-test. In addition, for each of the parameters, an analysis of variance (ANOVA) with the factors type of death (suicide, homocide), age group (≤35 years, >35 years), and sex (male, female) was performed. For each parameter, comparisons between the suicide and non-suicide means were made using Student's *t*-test with the variance estimate obtained from the three-factor analysis of variance [14].

Protein Determination

Protein concentrations were determined by the method of Bradford [6] with gamma globulin as a standard.

RESULTS

The results of ligand binding experiments were first analyzed in order to determine if age (≤35, >35 years) or sex influenced the data. Analysis of variance indicated that for each of the receptor systems studied, there were no differences between the two age or sex groups.

Specific ^{3}H-IMI binding was linear across a range (200–600 μg protein/tube) of human cortical tissue. At both 2.2 and 5.3 nM, ^{3}H-IMI binding was elevated in the suicide compared to the homicide group (Fig. 1, Table 1). The ratio of mean values of the suicide to homicide group (fmol/mg protein) was 1.35, indicating a 35% increase in specific binding. Analysis by Student's *t*-test indicated a *p*-value of ≤ 0.025 as shown in Table 1. When analyzed by ANOVA, to include age and sex parameters, a *p*-value of <0.04 was obtained.

Saturation binding assays for ^{3}H-QNB were analyzed by the Scatchard transformation, yielding values for receptor density (Bmax) and ligand affinity (Kd). Dissociation constants (Kd) were virtually identical for the two groups. However, Bmax values were greater (235 versus 155 fmol/mg protein) in the suicide group (Fig. 2, Table 1). This result was significant at the $p \leq 0.05$ level when analyzed by Student's *t*-test. A similar trend was apparent by ANOVA, $p < 0.096$.

For specific binding of ^{3}H-DHA to human cortical membranes, there were no significant differences between suicide and homicide groups at either ligand concentration (Table 1).

The percent of specific binding with ^{3}H-QNB was in excess of 90%. Considerably lower values were obtained for ^{3}H-IMI and ^{3}H-DHA, 35% and 25%, respectively. Fresh rat

TABLE 1

PARAMETERS OF SELECTED RADIOLIGAND BINDING TO HUMAN SUICIDE AND HOMICIDE CORTICAL MEMBRANES

	Specific Binding					
	^{3}H-QNB		^{3}H-IMI		^{3}H-DHA	
	Bmax (fmoles/mg prot)	Kd (nM)	2.2 nM (fmol/mg prot)	5.3 nM	0.5 nM (fmol/mg prot)	1.0 nM
Suicide	235 ± 36 (8)	0.066 ± 0.009 (8)	103 ± 8 (8)	172 ± 14 (8)	9 ± 2 (7)	18 ± 3 (7)
Homicide	155 ± 17 (10)	0.070 ± 0.004 (10)	75 ± 5 (10)	127 ± 9 (9)	8 ± 3 (9)	16 ± 2 (9)
p Value						
ANOVA	0.096	0.401	0.038	0.033	0.260	0.111
t-test	≤0.050		≤0.001	≤0.025		

Values shown are means ± S.E.M. Numbers in parentheses represent the n for each group.

cortical tissues assayed in parallel gave corresponding specific binding percentage values of 65% for ^{3}H-IMI and 70% for ^{3}H-DHA. We considered the possibility that postmortem degradation of the receptor had occurred either during the interval between death and tissue acquisition, or during the storage of frozen tissue prior to analysis. This possibility was diminished by our observation that receptor binding of the same ligands to freshly obtained human cortical tissue (<2 hours postmortem) yielded equivalent percentages of specific binding.

HPLC analysis of the samples revealed no detectable levels of imipramine, desipramine or amitriptyline at an assay sensitivity of 50 picograms/mg tissue.

DISCUSSION

The relative increase in specific muscarinic and imipramine binding in cortical tissue of suicides is most interesting given the tenuous relation between suicide and depression. Recent studies demonstrate that a decrease in ^{3}H-IMI sites [2,7] and 5-HT reuptake [11,22] in platelets derived from depressed patients may be predictive of a trait-dependent phenomenon and suggest that a similar profile exists in brain. Very similar biochemical characteristics do in fact exist between platelets and brain synaptosomes for ^{3}H-IMI binding [15,17] and 5-HT reuptake [19]. Clearly, a correlation does not exist between reported decreases in platelet ^{3}H-IMI binding in depressives and the increased

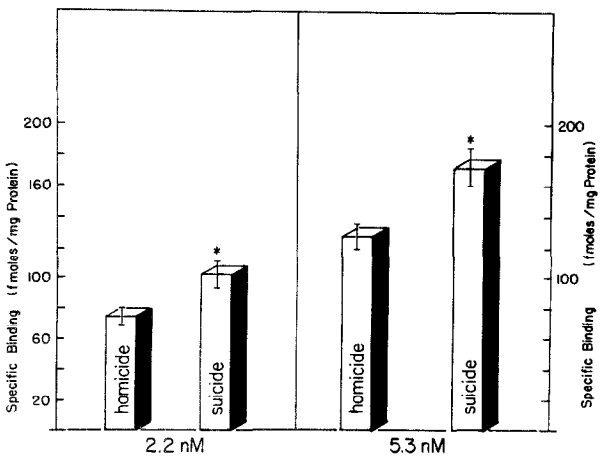

FIG. 1. Histogram of specific ^{3}H-imipramine binding in cortical tissues of suicide and homicide victims. Experiments were conducted at ligand concentrations of 2.2 and 5.3 nM. For details of tissue preparation and assay conditions see Method. Values shown represent means±S.E.M. *p≤0.04 (ANOVA); p≤0.001 at 2.2 nM and p≤0.025 at 5.3 nM (Student's t-test).

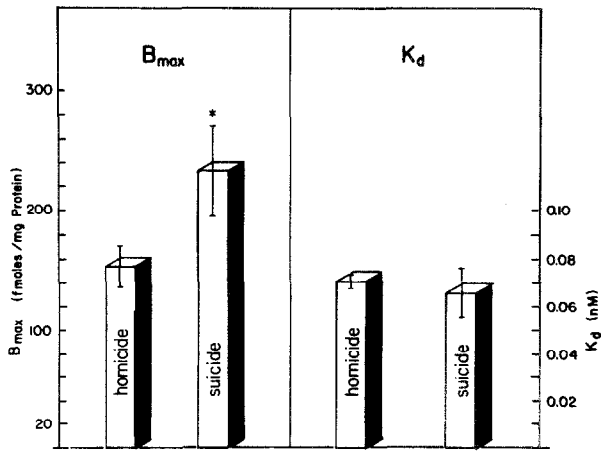

FIG. 2. Histogram of ^{3}H-QNB binding parameters in cortical tissues of suicide and homicide victims. Data are derived from Scatchard analysis of saturation isotherms and histogram results are the means of the plot X-intercepts (reflecting Bmax) and plot slopes (reflecting Kd). For details of tissue preparation and assay conditions see Method. Values shown represent means±S.E.M. *p=0.096 (ANOVA), p≤0.05 (Student's t-test).

binding of ^{3}H-IMI seen in the cortex of suicide victims. These data seem paradoxical assuming depressive illness is indeed a factor in suicidal behavior and the platelet is a model for neuronal function. To date, however, no investigations have demonstrated a decrease in ^{3}H-IMI recognition sites in neuronal tissue derived from brains of depressed subjects. Perhaps regulation of ^{3}H-IMI binding in platelets and neuronal tissue do not occur in concert.

One explanation for increased ^{3}H-IMI binding in suicide subjects is the alteration of endogenous biochemical modulators which stimulate both ^{3}H-IMI binding and the 5-HT reuptake process (Wennogle and Meyerson, unpublished observations). Alternatively, increased ^{3}H-IMI binding may represent a hyperactive 5-HT reuptake carrier or altered allosteric modifier of that carrier [8].

The relationship of suicide to the cholinergic hypothesis of depression is also provocative. Post-junctional cholinergic receptor supersensitivity, as evidenced by increased ^{3}H-QNB binding, may be due to a decreased cholinergic function (i.e., diminished synthesis or release) in suicide victims. The converse of this situation is suggested to exist in the depressive syndrome (i.e., cholinergic dominance) [12].

It is well established that chronic administration of tricyclic antidepressive agents produces marked modulations in rodent central nervous system neurotransmitter recognition sites [16]. For this reason, it was important to identify residual TAD's in the brains of the subjects in the study at the time of death. Although no TAD's were detected by a sensitive HPLC analysis, the possibility of prior treatment with these agents leading to an altered state of receptor sensitivity is not precluded.

It is necessary to comment on additional factors that may be responsible for the observed differences between homicide and suicide groups. In a study of this type, where it is impossible to control all past experience of the experimental subjects, certain unregulated behaviors may differ between the groups. These conditions, such as dietary alterations, sleep patterns or drug regimens and abuse, are potentially capable of producing the results obtained in these studies.

The cortical receptor alterations characterized in this study of suicide victims may be interpreted in terms of muscarinic and presynaptic serotonergic hypersensitivity. This state may either be related to disturbances in genetic receptor expression or compensatory to neurotransmitter function.

Finally, although suicide is often a consequence of depression, it must be emphasized that the receptor alterations observed in the brains of suicide victims in this study may not be exclusively associated with depression, since other psychopathological states also exhibit this behavioral tendency.

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