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Brain Noradrenergic Receptors in Major Depression and Schizophrenia

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The binding of [^{125}I]p-iodoclonidine to alpha-2, *and/or [^{125}I]iodopindolol to* beta-1 *and* beta-2 *adrenoceptors was measured in right prefrontal cortex (Brodmann's area 10) and right hippocampus from subjects with DSM-III-R diagnoses of major depression* (n = *15) or schizophrenia* (n = *8) as well as from control subjects* (n = *20). No significant differences between study groups were observed in binding to* alpha-2 *adrenoceptors in any of the six layers of prefrontal cortex or in any of the hippocampal fields. Likewise, there were no significant differences in* beta-1 *or* beta-2 *adrenoceptor binding in any of the hippocampal fields between control and major depressive subjects. In contrast, binding to* beta-1 *adrenoceptors, but not* beta-2 *adrenoceptors, was significantly lower (−13 to −27%) in most hippocampal fields of schizophrenic subjects as compared to control subjects or to major depressives. Alterations in* beta-1 *adrenoceptor binding in the hippocampus of schizophrenics provide further evidence for a role of central noradrenergic neurons in the neurochemical pathology of schizophrenia.*
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KEY WORDS: *Noradrenergic receptors; Schizophrenia; Depression; Prefrontal cortex; Hippocampus; Postmortem*

The central noradrenergic system serves a global function of neural modulation, controlling vigilance, attention, and the sleep–wake cycle and contributing to learning and memory processes. This system is also robustly activated by stress (Aston-Jones et al. 1991). Disruptions of attention, memory, and sleep are behavioral characteristics of major depression. Therefore, we could hypothesize that abnormal noradrenergic function could be responsible, at least in part, for these symptoms. The putative role that stress plays in depressive disorders and the ability of long-term stress to deplete norepinephrine lends credence to the hypothesis that there is pathological involvement of the central noradrenergic system in depression (Prange 1964; Schildkraut 1965; Ordway et al. 1994b; Klimek et al. 1997).

Studies designed to examine the noradrenergic hypothesis of affective disorders have been diverse and sometimes inconsistent (Prange 1964; Schildkraut 1965; Waldmeier 1981; Lake et al. 1982; De Paermentier et al. 1990; Ferrier and Perry, 1992; Meana et al. 1992; Gonzalez et al. 1994). Nevertheless, the majority of such reports support the idea that there is an abnormality of central catecholaminergic transmission in major depression (Prange 1964; Schildkraut 1965; Waldmeier 1981; Lake et al. 1982; Meana et al. 1992; Callado et al. 1998). Recently, Ordway et al. (1994a) reported elevated levels of tyrosine hydroxylase, the rate-limiting enzyme in the

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biosynthesis of norepinephrine, in the locus coeruleus, a major region of noradrenergic neuronal cell bodies, from victims of suicide. This finding has been confirmed in subjects diagnosed with major depression relative to psychiatrically normal control subjects (Zhu et al. 1995). Tyrosine hydroxylase in the locus coeruleus is also up-regulated in response to depletion of norepinephrine in rats (Melia et al. 1992) and in response to repeated stress (Melia and Duman 1991; Melia et al. 1992). Furthermore, Klimek et al. (1997) reported decreased binding of [^{3}H]nisoxetine to norepinephrine transporters in the locus coeruleus in major depression. This latter finding may also reflect a norepinephrine deficiency, given that these transporters down-regulate in rats following treatment with norepinephrine-depleting drugs (Lee et al. 1983). Thus, up-regulation of tyrosine hydroxylase and down-regulation of the norepinephrine transporter observed in postmortem studies are findings consistent with the hypothesis of a synaptic deficiency of norepinephrine in major depression.

Efferents of the locus coeruleus constitute two major ascending fiber systems; the dorsal noradrenergic bundle and the rostral limb of the dorsal periventricular pathway, that innervate subcortical limbic regions, such as the hippocampus, as well as most of the cerebral cortex. If a deficiency of norepinephrine exists in the locus coeruleus in major depression, then we would speculate that receptors for norepinephrine in projection areas of the locus coeruleus might be up-regulated in response. Numerous researchers have investigated the possibility of altered noradrenergic receptor densities in cortical areas over the past two decades, and the results have been inconclusive (Mann et al. 1986; Meana and Garcia-Sevilla 1987; Biegon and Israeli 1988; Arango et al. 1990; De Paermentier et al. 1990; Meana et al. 1992; Arango et al. 1993; Gonzalez et al. 1994; Callado et al. 1998). However, few of these studies have utilized tissue from subjects with confirmed diagnoses of major depression at the time of death, and fewer still have utilized control subjects who were confirmed as having no psychiatric illness prior to death.

In addition to a group of major depressives, we measured in the present study noradrenergic receptors in the hippocampus from subjects with another major psychiatric disorder; that is, schizophrenia. Schizophrenia was of particular interest, given evidence implicating a potential role of norepinephrine in the pathophysiology of this disorder. A disruption of the activity of the noradrenergic dorsal bundle from locus coeruleus to the forebrain limbic system could be a possible contributor to the schizophrenic disturbance (see Hornykiewicz 1986; Weinberger et al. 1983), given that activity of the dorsal bundle of the locus coeruleus normally serves to screen the incoming sensory stimuli, filtering out and discarding information irrelevant to the task at hand (Archer et al. 1982; Mason 1981). Evidence that norepinephrine regulates dopamine-induced behaviors in laboratory animals (Mason 1981; Plaznik et al. 1982) provides a potential link of norepinephrine to dopaminergic dysfunction in schizophrenia. Interestingly, elevated norepinephrine output has been observed in schizophrenic patients (Lake et al. 1980; Sternberg et al. 1981; Hornykiewicz 1982, 1986; Van Kammen and Antelman 1984).

In this study, we measured *alpha-2*, *beta-1*, and *beta-2* adrenoceptors autoradiographically in the prefrontal cortex and/or hippocampal formation from subjects with Axis I diagnoses of major depression or schizophrenia relative to control subjects who had no Axis I diagnoses (confirmed by psychiatric autopsy) and control subjects with no reported history of psychiatric illness and no psychiatric medications. Most of the psychiatric subjects in this study died as a result of suicide; whereas, control subjects died of natural or accidental causes.

MATERIALS AND METHODS

Tissue Material

Human brains were obtained from subjects at the time of autopsy at the Medical Examiner's Office of Cuyahoga County, Ohio, in accordance with an approved Institutional Review Board protocol. Subjects were coded to protect their identities. Causes of death were determined by the coroner. All subjects were refrigerated before autopsy once arriving at the coroner's office. Information on the lifetime and current (within last month) psychiatric status of all psychiatric subjects and most control subjects (see below) was obtained in structured clinical interviews by a trained interviewer with the next of kin. The interview used was the Schedule for Affective Disorders and Schizophrenia: Lifetime version (SADS-L, Endicott and Spitzer 1978) supplemented by questions from the Diagnostic Interview Schedule (DIS-III-R) (Robbins et al. 1989) to make diagnoses compatible with the Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R, American Psychiatric Association 1987). The SADS has obtained adequate validity when comparing the patient report to that of an informant (Andreasen et al. 1977). Recently, Kelly and Mann (1996) have validated the use of psychological autopsy by demonstrating good agreement between informant-based retrospective psychological assessment of deceased subjects and chart diagnoses generated by clinicians treating the same subjects before death. They reported a 91% agreement between DSM-III-R diagnoses made antemortem by treating clinicians and informant-based diagnoses made postmortem. Evaluation of drug and alcohol abuse and dependency was assessed using the DIS-III-R. Axis I diagnoses were made by a psychiatrist (H.Y.M.) and a clinical psychologist (J.C.O.), based on data gathered from the structured

Table 1. Psychiatric Subject Demographics

Study No.	Death	Age	Race/Sex	Cause of Death	PMI	Toxicology	Axis I
1,2,3	Suicide	25	w/f	Hanging	17	NDD	Major depression
1,2,3	Suicide	29	w/m	Hanging	9	Cocaine, etoh	Major depression, alcohol dependence, cocaine depend.
1,2,3	Suicide	38	w/f	OD	12	Etoh, diazepam, temazepam, lidocaine, acetaminophen	Major depression
1,2,3	Suicide	42	w/m	SIGSW	20	NDD	Major depression, single episode
1,2,3	Suicide	43	w/m	Hanging	21	NDD	Major depression
1,2,3	Suicide	45	w/m	Multiple knifing	8	NDD	Major depression, dysthymia
1,2,3	Suicide	47	w/m	SIGSW	11	Etoh	Major depression
1,2,3	Suicide	62	w/m	SIGSW	20	NDD	Major depression
2,3	Suicide	62	w/m	Hanging	5	NDD	Major depression, smoker
1,2,3	Natural	63	w/f	Heart	18	Lidocaine	Major depression, smoker, diabetes
1,2,3	Suicide	68	w/m	CO	4	NDD	Major depression, single episode, Parkinson's disease
1,2	Suicide	73	w/m	SIGSW	18	Diazepam, codeine	Major depression
2,3	Suicide	75	w/f	CO	30	NDD	Major depression, alcohol dependence
1,2,3	Suicide	83	w/f	Slashed wrists	21	NDD	Major depression
1,3	Suicide	70	b/m	SIGSW	23	Phenytoin (ER)	Major depression
2,3	Suicide	23	b/m	Hanging	19	NDD	Schizophrenia
2,3	Natural	31	w/f	Heart	29	Lidocaine	Schizophrenia
2,3	Suicide	32	b/m	SIGSW	10	NDD	Schizophrenia
2,3	Natural	41	b/m	Heart	16	Lidocaine, cocaine	Schizophrenia, inhalent dependence
2,3	Natural	43	w/f	Heart	21	NDD	Schizophrenia
2,3	Suicide	43	w/f	Asphyxia	10	NDD	Schizophrenia, anorexia nervosa
2,3	Suicide	51	w/m	SIGSW	18	NDD	Schizophreniz, smoker
2,3	Natural	53	w/m	Heart	12	Phenothiazine metabolites	Schizophrenia

Key: Study no.: (1) — [^{125}I]PIC binding in prefrontal cortex; (2) — [^{125}I]PIC binding in the hippocampus; (3) — [^{125}I]IPIN binding in the hippocampus; PMI — postmortem interval; natural —natural death; heart — death as a result of cardiovascular problems; etoh — ethanol; SIGSW — self-inflicted gun-shot wound; NDD — no drugs detectable; OD — overdose; CO — carbon monoxide poisoning; Axis I — DSM-III-R diagnosis.

interview and, when available, hospital and doctor's records. No psychiatric information could be obtained for nine of the 20 control subjects. Although there was no evidence in the coroner's records of any history of psychiatric or neurological disease or prescription medications for psychiatric illnesses in these nine control subjects, the lack of psychiatric illness could not be verified through structured interviews.

Brain tissues were collected from 15 subjects diagnosed of major depression, eight subjects with schizophrenia (summary of subject information is outlined in Table 1) and 20 control subjects. The age of subjects ranged from 23 to 83 years, and demographic comparisons for all three groups of subjects is given in Table 2. The age of control subjects was 52 ± 4 (mean ± SEM) year, major depressives 54 ± 5 year, and schizophrenics 40 ± 3 year. Postmortem delay was 19 ± 1 h for control subjects, 15 ± 2 h for major depressives, and 16 ± 3 h for schizophrenics. Among the 15 subjects diagnosed of major depression, two were diagnosed with co-morbid alcohol dependence, two were smokers (smoking more than 1 pack/day), and one had cocaine dependence. One major depressive had been diagnosed with Parkinson's disease. Among eight schizophrenics, one had a co-morbid diagnosis of inhalant dependence, one of anorexia nervosa, and one was a smoker. Subjects in the control group consisted of six females and 14 males, and the causes of death in this group were: cardiovascular failure (n = 13), gunshot (n = 1), pulmonary embolism (n = 1), aneurism (n = 2), pancreatitis (n = 1), thrombophlebitis (n = 1), and bicycle accident (n = 1). Eleven control subjects were assessed retrospectively through structured interviews and had no Axis I diagnosis (DSM-III-R). One of these control subjects had a history of an episode of adjustment disorder with depressed mood 5 months before death. The remaining nine control subjects had no evidence of psychiatric his-

Table 2. Demographic Comparisons By Group

	n	Age	PMI	W/B	M/F	Suicides
Normal control	20	52 ± 4	19 ± 1	16/4	14/6	0
Major depression	15	54 ± 5	15 ± 2	14/1	10/5	14
Schizophrenia	8	40 ± 3	16 ± 3	5/3	5/3	4

The values for age and PMI (postmortem delay) represent: mean ± SEM.

tory or history of psychiatric medication, according to the coroner's records.

A toxicology screen of blood, bile, and urine from all subjects was performed by the county coroner's office. Qualitative and quantitative assays were used to detect the following compounds or classes of compounds: ethanol, barbiturates, benzodiazepines, sympathomimetic drugs, and antidepressant and antipsychotic drugs and their metabolites. In the course of collecting tissue for these studies, all subjects with evidence of antidepressant drugs, other psychotherapeutic drugs, or other psychoactive compounds in the toxicology screen were not included in the study. Toxicology results of major depressive subjects and schizophrenics are shown in Table 1. The toxicology screen of control subjects revealed the following: one subject had alcohol (0.07%) in the blood, two had chlorpheniramine, one had ephedrine, and four had lidocaine. Records collected did indicate antidepressant drug prescriptions within the last month for two of the subjects with major depression and antipsychotic drugs for four of the subjects with schizophrenia.

Dissection

At the time of autopsy, brain tissue was dissected into small blocks. Particular care was taken in the freezing process to maintain gross morphology. Tissues containing right prefrontal cortex (Brodmann's area 10) and right hippocampus, respectively, were placed on hard cardboard, then dipped in isopentane (−50°C) for 5 seconds. Tissues were placed on dry ice for 10 min and then stored at −82°C. The location of Brodmann's area 10 was confirmed cytoarchitectonically (Rajkowska and Goldman-Rakic 1995a,b; Rajkowska et al. 1998). Hippocampal fields (Figure 1) were outlined based on morphological criteria (Duvernoy 1988). Frozen blocks of prefrontal cortex and the right hippocampus were mounted on a specimen chuck of a cryostat microtome (Leica, Cryocut 1800, Reichert-Jung), and tissue sections (20-μm thick) were cut at −16°C and thaw-mounted onto gelatin-coated microscope slides. Sections were dried under refrigeration and then stored at −82°C until assay. Adjacent sections (40-μm thick) were cut for morphometry, dried at room temperature, and then stained with cresyl violet.

Quantitative Autoradiography

Quantitative autoradiography of the binding of p-[^{125}I]-iodoclonidine ([^{125}I]PIC) to *alpha-2* adrenoceptors was performed according to a previously published method (Ordway et al. 1994b). Briefly, duplicate sections were preincubated in Tris-Mg buffer (170 mM Tris, 10 mM $MgCl_2$, pH 7.6) at 23°C for 60 min. Sections were then transferred into slide mailers containing 6 ml of Tris-Mg buffer plus 300 pM of [^{125}I]PIC (2,200 Ci/mmol; New England Nuclear, Wilmington, DE) and incubated for 90 min followed by a 10-min wash in ice-cold Tris buffer. Nonspecific binding of [^{125}I]PIC was defined in the presence of 10 μM *l*-norepinephrine. The use of norepinephrine to define nonspecific binding eliminated the influence of imidazoline sites in the calculation of specific binding to *alpha-2* adrenoceptors.

The binding of [^{125}I]iodopindolol ([^{125}I]IPIN) to *beta-1* and *beta-2* adrenoceptors was performed, as previously described (Ordway et al. 1988). Duplicate sections were incubated in Tris buffer (Tris 20 mM; NaCl 135 mM, pH 7.4) containing 250 pM of [^{125}I]IPIN for 120 min at 22°C

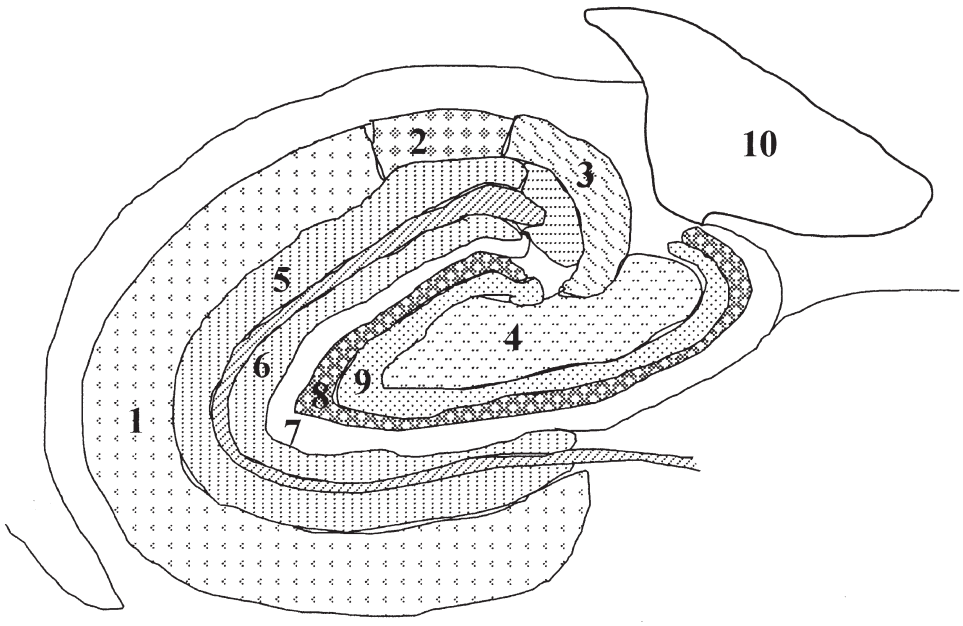

Figure 1. Structure of the human hippocampus in coronal section (according to (Duvernoy 1988). This template was used to align hippocampal fields in cresyl violet-stained sections with autoradiograms of the binding of radioligands. 1 = CA1, 2 = CA2, 3 = CA3, 4 = CA4 (fields of cornu ammonis); 5 = C1R (stratum radiatum of cornu ammonis); 6 = C1M (stratum moleculare of cornu ammonis); 7 = MOL (stratum moleculare of detate gyrus); 8 = GRN (stratum granulosum); 9 = POL (polymorphic layer); 10 = FIM (fimbria). Inner and outer layers of the molecular layer (IM and OM) are not shown.

in the presence of the *beta-2* antagonist, ICI 118,551 (50 nM) or the *beta-1* antagonist, ICI 89,406 (70 nM), respectively. Sections were then washed in ice-cold buffer for 60 min. The nonspecific binding of [^{125}I]IPIN was determined in the presence of *l*-isoproterenol (100 μM). Dry sections and brain mash calibrated standards (American Radiolabeled Chemicals, Inc., St. Louis, MO) were apposed to [^{3}H]Ultrofilm (Leica Instruments, GmbH) and exposed in X-ray cassettes at room temperature for 20 h. Films were processed with GBX developer and fixer (Eastman Kodak, Rochester, NY) at 17°C. After autoradiography, the same sections were stained lightly with cresyl violet for the anatomical identification. All autoradiograms were analyzed by simultaneously overlaying the image of the autoradiogram with the image of the same Nissl stained section. Densitometric measurements of autoradiograms were made using the Microcomputer Controlled Imaging Device (MCID M2, Imaging Research Inc., St. Catherines, Ontario, Canada).

Statistics

Binding data were analyzed by analysis of variance (ANOVA) for repeated measures (several brain regions in each subject). Planned comparisons were made using contrast analyses. Linear regression analyses were used to compute correlations between binding and age or postmortem interval (GraphPad Prism Ver. 1.0, GraphPad Software Inc., San Diego, CA). Data are reported as the means ± the standard error of the mean.

Abbreviations

CA1, CA2, CA3, CA4 = fields of the cornu ammonis of the hippocampus; C1R = stratum radiatum of cornu ammonis; C1M = stratum moleculare of cornu ammonis; MOL = stratum moleculare of dentate gyrus (IM = inner; OM = outer part of molecular layer); GRN = stratum granulosum; POL = polymorphic layer; FIM = fimbria.

RESULTS

Autoradiography of *Alpha*-2 Adrenoceptors

The binding of [^{125}I]PIC to *alpha-2* adrenoceptors was measured within 11 fields of the hippocampus from 20 control subjects, 14 subjects with a diagnosis of major depression and eight with a diagnosis of schizophrenia, as well as in each of six layers of prefrontal cortex from 13 control subjects and 13 major depressives.

Binding of [^{125}I]PIC to *alpha-2* adrenoceptors in hippocampal fields showed a distinct laminar pattern in MOL (Figure 1), with binding in IM much greater than OM (Figure 2). The highest binding of [^{125}I]PIC to *alpha-2* adrenoceptors within the hippocampus was observed in the C1M and IM, and the lowest binding was in the CA2, CA1, and C1R regions. Specific binding was nearly absent in the FIM (Figure 2). By overlaying the autoradiograms with their respective Nissl-stained sections, the other laminae that did not appear as dense autoradiographic bands could also be identified.

The specific binding of [^{125}I]PIC in hippocampal fields from control, major depressive and schizophrenic subjects revealed identical laminar patterns of *alpha-2* adrenoceptor distributions. Furthermore, no significant differences in [^{125}I]PIC binding densities were observed in any of the hippocampal fields between the study groups of control subjects and subjects having a diagnosis of major depression or schizophrenia (Figure 2).

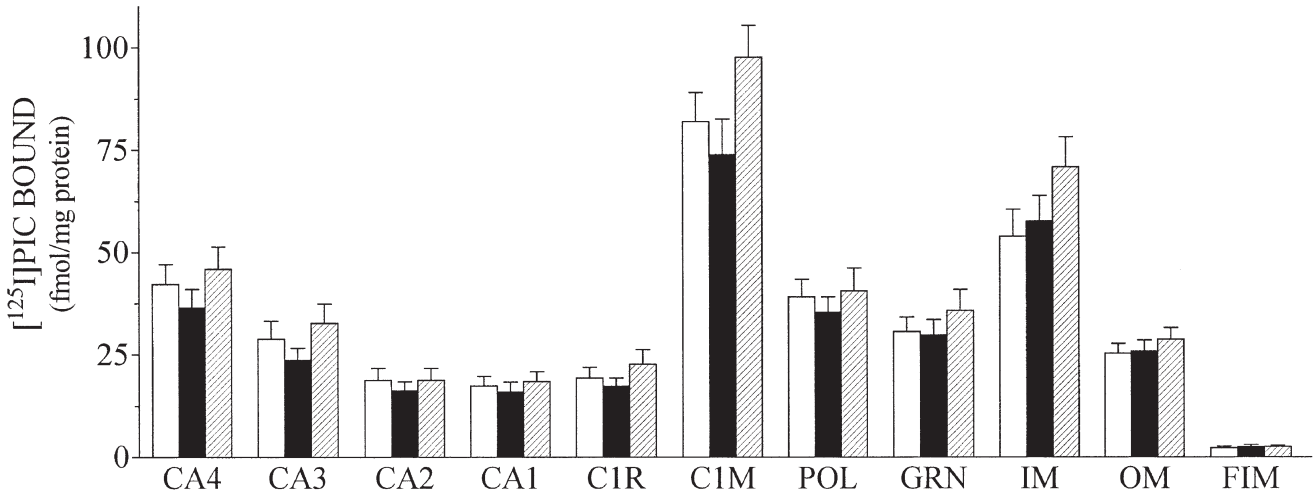

Figure 2. Specific binding of [^{125}I]PIC to *alpha-2* adrenoceptors in hippocampal fields of 14 subjects diagnosed with major depression (filled bars), eight subjects with schizophrenia (striped bars) and 20 control cases (open bars).

In the prefrontal cortex (Brodmann's area 10), the distribution of [^{125}I]PIC binding to *alpha-2* adrenoceptors was not uniform among six cortical layers (Figure 3). The highest binding of [^{125}I]PIC to *alpha-2* adrenoceptors was observed in layers III and IV. Similar to that observed in the hippocampus, there were no significant differences in [^{125}I]PIC binding to *alpha-2* adrenoceptors between subject groups (Figure 3).

Although control and major depressive subjects were matched by age, postmortem delay, and gender as closely as possible before experimentation, we found no significant correlation in either study group between age or postmortem delay and the binding of [^{125}I]PIC to *alpha-2* adrenoceptors in any of the 11 subregions of hippocampus or in any of the six layers of the cerebral cortex (data not shown).

Autoradiography of *Beta-1* and *Beta-2* Adrenoceptors in the Hippocampus

Autoradiograms of [^{125}I]IPIN binding to *beta-1* and *beta-2* adrenoceptors showed distinct laminar distribution throughout the hippocampal formation (Figures 4–6). However, in contrast to [^{125}I]PIC binding to *alpha-2* adrenoceptors, IM and OM of the MOL were not distinguishable. The highest amount of [^{125}I]IPIN binding to *beta-1* adrenoceptors was observed in CA1, C1R, C1M, and MOL subfields of the hippocampus (Figures 4 and 5).

There were no significant differences in the binding of [^{125}I]IPIN to *beta-1* adrenoceptors in any of the subfields of the hippocampus comparing major depressive subjects to control subjects. However, there was significantly lower [^{125}I]IPIN binding to *beta-1* adrenoceptors in several fields of the hippocampus in schizophrenic subjects as compared to control subjects and to major depressive subjects (Figures 4 and 5). The largest differences between schizophrenic and control subjects in [^{125}I]IPIN binding to *beta-1* adrenoceptors occurred in the C1R sector (-27%; $p < .001$), with slightly smaller differences occurring in CA1 (-22%; $p < .01$), POL (-20%; $p < .05$), GRN (-19%; $p < .01$), MOL (-19%; $p < .01$), and C1M (-19%; $p < .05$). The smallest, but statistically significant, decrease in binding was observed in CA4 (-15%; $p < .05$) and CA3 (-16%; $p < .05$; Figure 5). In the FIM, the binding of [^{125}I]IPIN to *beta-1* adrenoceptors was the lowest and was not significantly different between subjects of different study groups (Figure 5). Although records collected did indicate antipsychotic drug prescriptions within the last month before death for four schizophrenic subjects, levels of *beta-1* adrenoceptor binding in these subjects were not different from that in the other four schizophrenic subjects (data not shown).

Suicide was the cause of death for most of the subjects diagnosed with major depression and schizophrenia. Therefore, the binding of [^{125}I]IPIN to *beta-1* adrenoceptors in the subfields of the hippocampus of suicides (including major depressive and schizophrenic subjects; $n = 16$) was compared to that of control subjects ($n = 19$) and no significant differences were observed (data not shown).

The specific binding of [^{125}I]IPIN to *beta-2* adrenoceptors was about three times higher than its binding to *beta-1* adrenoceptors in the same hippocampal subregion and showed a different pattern of laminar distribution than the *beta-1* adrenoceptor distribution. Highest binding of [^{125}I]IPIN to *beta-2* adrenoceptors was observed in C1M, POL, GRN, MOL of the hippocampus (Figure 6). There were no significant differences in binding to *beta-2* adrenoceptors between the three groups of subjects for any of the subregions of the hippocampus (Figure 6). Despite the lack of statistical significance, amounts of [^{125}I]IPIN to *beta-2* adrenoceptors were consistently lower in all hippocampal subregions of the schizophrenic subjects relative to control and major depressive subjects.

Similarly to the studies of *alpha-2* adrenoceptors, the control, schizophrenic, and major depressive subjects

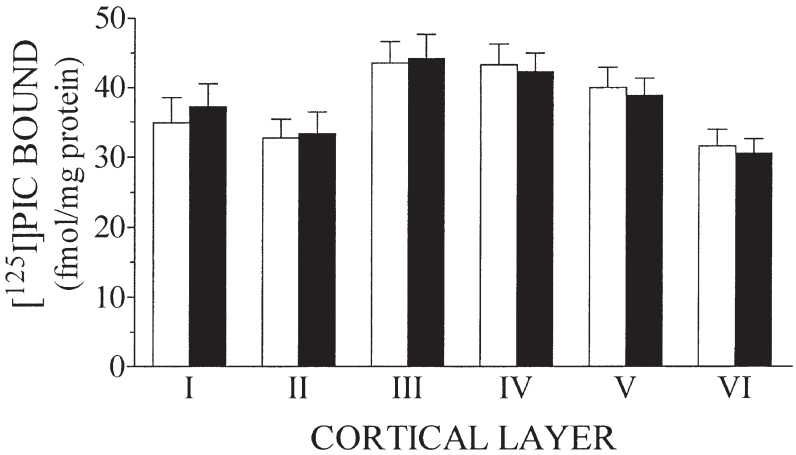

Figure 3. Specific binding of [^{125}I]PIC to *alpha-2* adrenoceptors in six layers of prefrontal cortex (Brodmann's area 10) from 13 age-matched pairs of major depressive (filled bars) and control subjects (open bars).

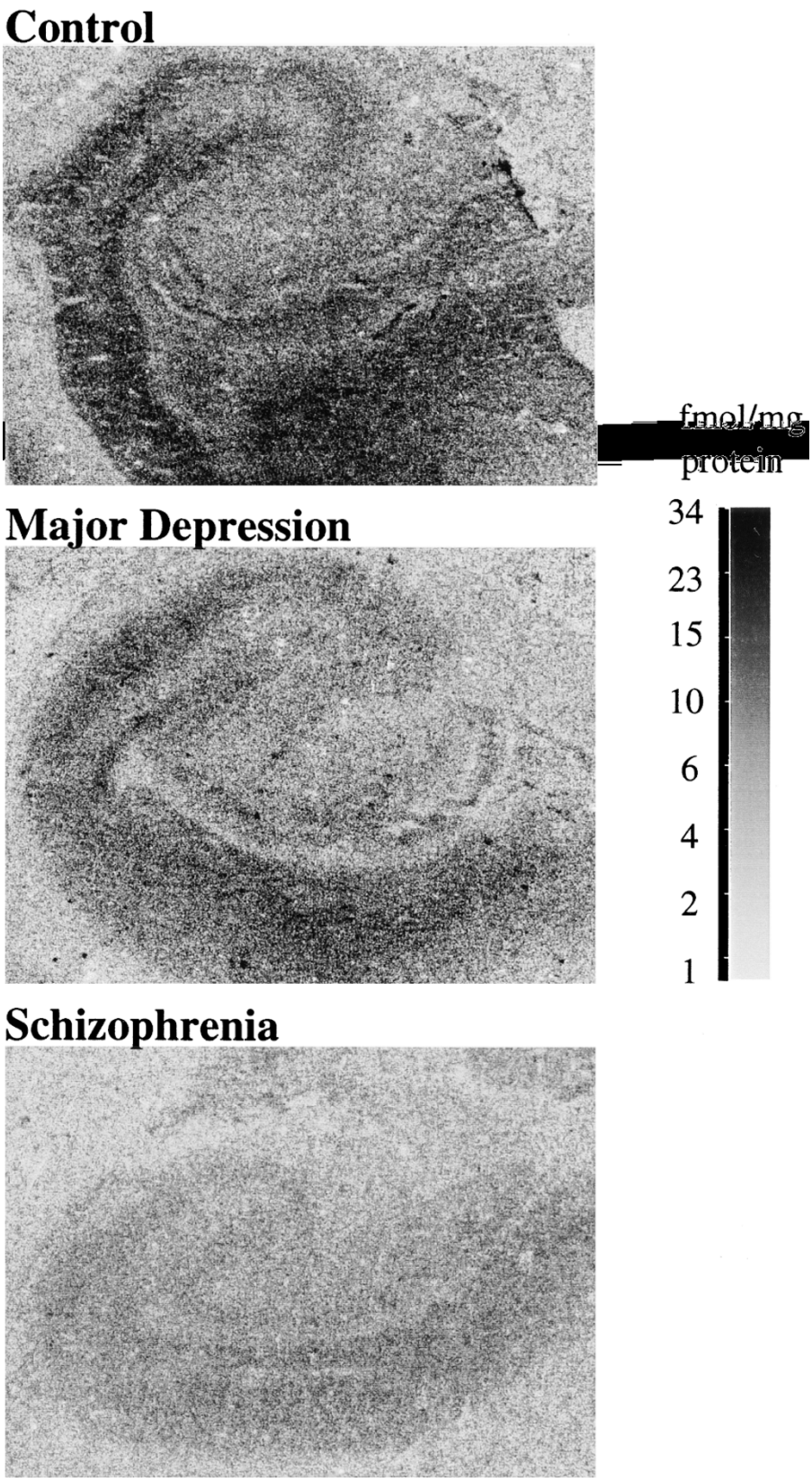

Figure 4. Digitized autoradiograms of the specific binding of [^{125}I]IPIN to *beta-1* adrenoceptors in hippocampal fields of a representative of control, major depressive, and schizophrenic subjects.

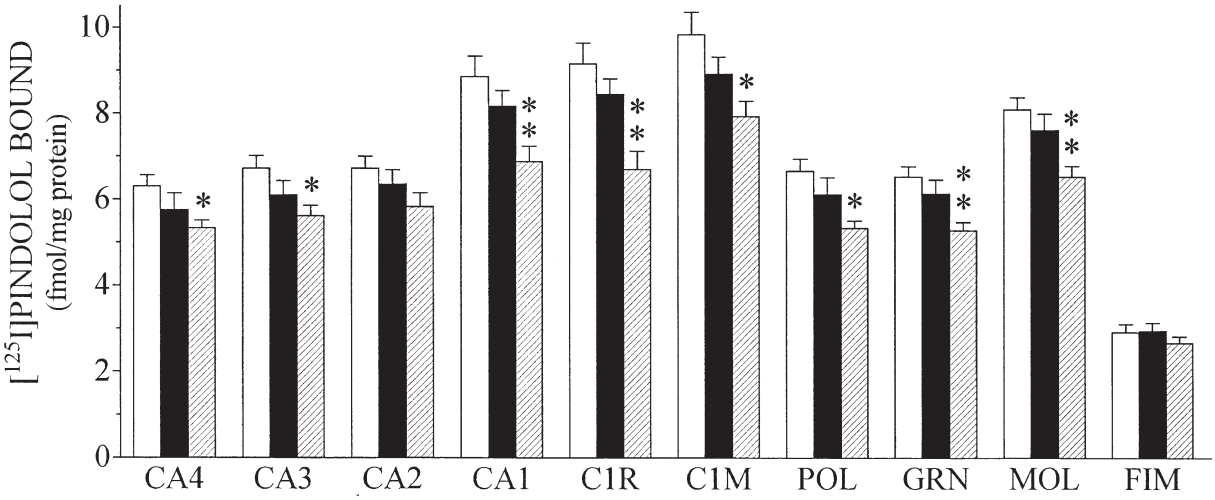

Figure 5. Specific binding of [^{125}I]IPIN to *beta-1* adrenoceptors in hippocampal fields of 15 subjects diagnosed with major depression (filled bars), eight subjects with schizophrenia (striped bars), and 20 control subjects (open bars). Significant difference from the control group; *$p < .05$, **$p < .01$.

were matched by age, postmortem delay, and gender as closely as possible before experimentation, we found no significant correlation in either study group between age or postmortem delay and the binding of [^{125}I]IPIN to neither *beta-1* nor *beta-2* adrenoceptors in any of the 11 fields of hippocampus (data not shown).

DISCUSSION

Noradrenergic receptors in the brain of laboratory animals are up- or down-regulated in response to decreases or increases, respectively, in synaptic norepinephrine (Bylund 1979; Waldmeier 1981; Bylund 1988; Barturen and Garcia-Sevilla 1992; Kovachich et al. 1993). The plasticity of noradrenergic receptor density has led a number of investigators to measure noradrenergic receptors in postmortem tissues from psychiatrically ill subjects in an attempt ascertain the premortem neurochemical status of noradrenergic transmission. After almost two decades of research, there is no consensus among researchers with regard to whether noradrenergic receptors are not changed, up- or down-regulated in psychiatric disease (Crow et al. 1984; Meana et al. 1992; Arango et al. 1993; Gonzalez et al. 1994; Ordway et al. 1994b; Callado et al. 1998). There are many possible explanations for the lack of consensus, but primary among them are the heterogeneity in psychiatric

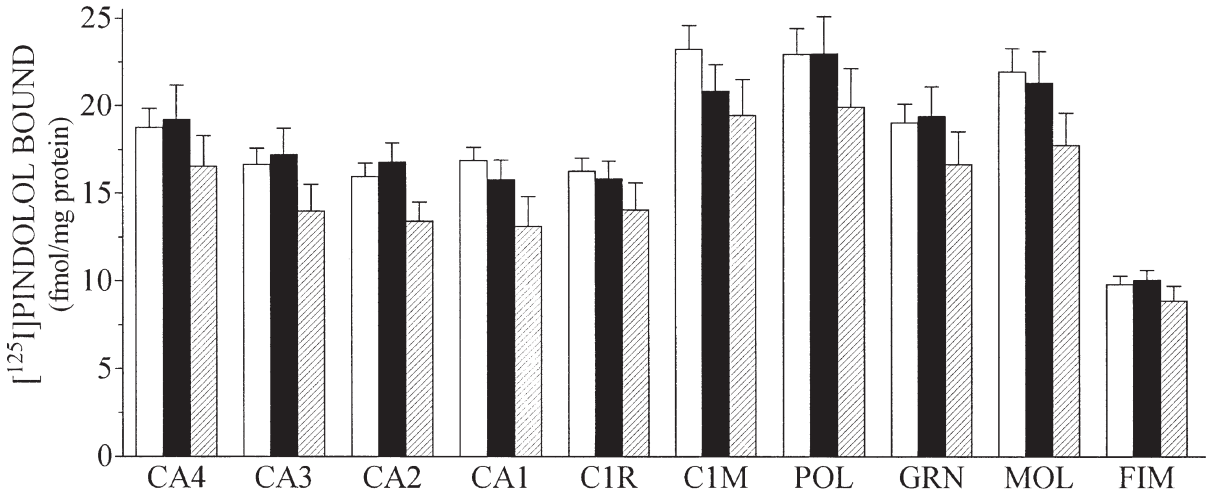

Figure 6. Specific binding of [^{125}I]IPIN to *beta-2* adrenoceptors in hippocampal fields of 15 subjects diagnosed with major depression (filled bars), eight subjects with schizophrenia (striped bars) and 20 control subjects (open bars).

illnesses of subjects within study groups and lack of precise anatomical location of measurements (Rajkowska 1997). The present study was designed to reduce variability in the sample population and to address more directly the role of the noradrenergic system in major depression and schizophrenia by: (1) using tissues from individuals for whom a rigorous evaluation of psychiatric status was performed for both control and psychiatric cases; (2) applying strict exclusion criteria to reduce or eliminate the contribution of neuropathology and recent antidepressant drug treatment; (3) confirming anatomical designations using cytorarchitectonic criteria; and (4) careful pairing and simultaneous processing of tissues from control and psychiatric subjects. Our findings confirm the findings of some, but not all, researchers demonstrating no differences in levels of binding to *alpha-2* or *beta* adrenergic receptors in cortical or subcortical brain regions in subjects with major depression as compared to psychiatrically normal control subjects. Unexpectedly, reduced binding to *beta-1* adrenergic receptors in the hippocampus of schizophrenic subjects suggests that greater attention should be paid to the study of the noradrenergic system in this illness.

Alpha-2 Adrenoceptors

Our data reveal no differences in the amount of [^{125}I]-PIC binding to *alpha-2* adrenoceptors measured in regions of the hippocampal formation or in any of cerebral cortical layers when comparing subjects with diagnoses of major depression or of schizophrenia to psychiatrically normal controls. Moreover, no change was observed in the binding of *alpha-2* adrenoceptor antagonists; [^{3}H]RX 781094 in the frontal cortex (Ferrier et al. 1986; Callado et al. 1998) or [^{3}H]rauwolscine in the hippocampus and occipital cortex (Crow et al. 1984) of subjects diagnosed with major depression in comparison to the control cases. Because most of the major depressives in the present study were victims of suicide, these data are concordant with the observations of Arango et al. (1993), who found no difference in binding of the related *alpha-2* adrenoceptor agonist, [^{3}H]p-aminoclonidine, to *alpha-2* adrenoceptors in the prefrontal cortex of victims of suicide relative to the normal controls. In contrast, another group of researchers (Gonzalez et al. 1994; Callado et al. 1998) have demonstrated a higher number of binding sites for [^{3}H]UK 14304 and for [^{3}H]clonidine (Callado et al. 1998), agonists at *alpha-2* adrenoceptors, in the hippocampus and frontal cortex (Brodmann's area 8 and 9) of depressed suicide victims compared to controls who were free of medical diagnosis and treatment. The discrepancies between our data and those of Garcia-Sevilla group (Gonzalez et al. 1994; Callado et al. 1998) could be explained by different anatomical localizations studied within prefrontal cortex. Brodmann's area 10 (studied in the present paper) could be clearly distinguished from more posteriorly located areas 8 and 9 by a distinct cytoarchitectonic and myeloarchitectonic pattern of cortical composition (Rajkowska and Goldman-Rakic 1995a, 1995b). Although Arango and co-workers (1993) did not report the psychiatric status of the suicide victims, they did not observe any changes in binding to *alpha-2* adrenoceptors in the anterior part of Brodmann's area 9. Because the most anterior part of area 9 (studied by Arango et al. 1993) has some cytoarchitectonic features similar to those of area 10 (present study; Rajkowska and co-workers, unpublished observations), it is possible that the same cytoarchitectonic region was analyzed in both studies.

Another discrepancy between the studies of the Garcia-Sevilla group (Gonzalez et al. 1994; Callado et al. 1998), Arango et al. (1993), and the present investigation was the length of the postmortem delay for the tissue specimens. The average postmortem delays for the present study were 15 $\pm$ 2 h for major depressives. The postmortem delays for the study by Arango et al. (1993) was 15 $\pm$1 h for suicide subjects. The postmortem delays for psychiatric cases by the Garcia-Sevilla group were more than twice as long as Arango et al.(1993) and the present study. For example, Gonzalez et al. (1994) reported postmortem delays of 36 $\pm$ 16 h for depressed suicides, and Callado et al. (1998) reported postmortem delays of 39 $\pm$ 6 h for depressed suicides. Interestingly, the only other report of elevated agonist ([^{3}H]UK 14304) binding to *alpha-2* adrenoceptors in human frontal cortex was by Meana et al. (1992), who also utilized tissues that had an average postmortem delay of approximately 30 hours for depressed suicides. None of the studies cited above, including the present investigation, demonstrated a significant effect of postmortem delay on levels of binding to *alpha-2* adrenoceptors. However, it should be kept in mind that one variable in studies utilizing tissues from depressed suicide victims is the environmental conditions surrounding the death. Because suicide is rarely performed in a public setting, the victim is often found hours after the suicide. Hence, it is conceivable that variability in the environmental conditions surrounding the victim during this time, before the cadaver is refrigerated, could produce highly variable effects on postmortem decay that could make difficult the demonstration of statistically significant correlations between postmortem delay and binding. Carefully controlled experiments designed to mimic environmental conditions occurring in the postmortem interval are difficult, but are warranted to rule out the possibility that long delays "unmask" radioligand binding to *alpha-2* adrenoceptors.

There are three subtypes of *alpha-2* adrenoceptors in the brain (Bylund 1985; Bylund 1988; Lomasney et al. 1990; Harrison et al. 1991; Nicholas et al. 1993). The presynaptic *alpha-2* adrenoceptor on noradrenergic neu-

rons; that is, the autoreceptor, seems to be the *alpha-2A* adrenoceptor (De Vos et al. 1992; Nicholas et al. 1993). However, this subtype of adrenoceptor also occurs postsynaptically on a number of catecholaminergic and noncatecholaminergic neurons, as well as on glia in many brain regions, including the prefrontal cortex (Aoki et al. 1994). Most of the *alpha-2* adrenoceptors in a projection area such as frontal cortex are post-, rather than presynaptic (autoreceptors), because lesioning noradrenergic neuronal fibers in the rat brain does not decrease binding of [^{3}H]clonidine (U'Prichard et al. 1979; Dausse et al. 1982; Gross et al. 1985) or [^{3}H]rauwolscine (Ordway 1995) to *alpha-2* adrenoceptors in the cerebral cortex. Thus, it is likely that the synaptic location of *alpha-2* adrenoceptors labeled in the present study was predominately postsynaptic. Furthermore, it is likely that the *alpha-2A* adrenoceptor is the predominate subtype labeled in the present study, because this subtype is the predominate *alpha-2* adrenoceptor in the human frontal cortex (De Vos et al. 1992; Ordway et al. 1993; Callado et al. 1998), and [^{125}I]PIC binds this subtype preferentially (Gerhardt et al. 1990; Ordway et al. 1993; Ordway et al. 1994b).

Beta Adrenoceptors

The binding of [^{125}I]IPIN to *beta-1* and *beta-2* adrenoceptors in the hippocampus of major depressive subjects was not significantly different from that of normal control subjects. These data are concordant with another study (De Paermentier et al. 1990) that demonstrated no differences in *beta-1* and *beta-2* adrenoceptor densities, using the radioligand, [^{3}H]CGP 12177, in the hippocampus of control subjects and depressed suicide victims. In contrast, Crow and co-workers (1984) observed a decrease in the binding of [^{3}H]dihydroalprenolol to *beta* adrenoceptors (*beta-1* plus *beta-2*) in the hippocampus of depressed subjects as compared to control cases. In this latter study, the nonspecific binding of [^{3}H]dihydroalprenolol was determined with propranolol. In addition to beta adrenoceptors, both propranolol and [^{3}H]dihydroalprenolol label serotonin receptors. According to the studies of Riva and Creese (1989), there is no concentration of propranolol that could satisfactorily be used to displace [^{3}H]dihydroalprenolol specifically from *beta* adrenoceptors. Hence, it is difficult to know whether lower binding in the hippocampus of depressive subjects observed by Crow and co-workers (1984) is a decrease in *beta* adrenergic or serotonin receptors.

The binding of [^{125}I]IPIN to *beta-1* adrenoceptors was approximately threefold lower than binding to *beta-2* adrenoceptors. Furthermore, the pattern of distribution of *beta-1* adrenoceptors throughout the hippocampus was distinctly different from that of *beta-2* adrenoceptors. The high concentration of *beta-2* adrenoceptors, relative to *beta-1* adrenoceptors, in the human hippocampus is in marked contrast to the relative densities of these receptors in the rat hippocampus (Rainbow et al. 1984). Joyce et al. (1992) have also demonstrated a high density of *beta-2* adrenoceptors relative to *beta-1* adrenoceptors in the human hippocampus.

Of major interest in the present study is the demonstration of lower [^{125}I]IPIN binding to *beta-1* adrenoceptors in the hippocampus from schizophrenic subjects relative to subjects with major depression or psychiatrically normal control subjects. To the best of our knowledge, this is the first demonstration of reduced radioligand binding to *beta-1* adrenoceptors in hippocampal formation of schizophrenics. Joyce and co-workers (1992), using the same radioligand and displacing agents, reported no statistically significant differences in *beta-1* or *beta-2* adrenoceptor binding in the hippocampus between control and schizophrenic subjects. Joyce and co-workers (1992) studied the field of the cornu ammonis in both left and right hippocampi. Examination of their data from the right hippocampus shows 25% lower binding to *beta-1* adrenoceptors in schizophrenic subjects as compared to control subjects. Although this difference did not reach statistical significance (Joyce et al. 1992), it is comparable to the magnitude of reductions observed in the present study in which all hippocampi were obtained from the right hemisphere, and in which discrete fields of the cornu ammonis were measured.

Reduced binding of [^{125}I]IPIN to hippocampal *beta-1* adrenoceptors may reflect receptor down-regulation secondary to enhanced noradrenergic transmission or may be a result of a general degenerative process in schizophrenia. The postulate of noradrenergic overactivity in schizophrenia is supported by a number of observations. For example, hippocampal *beta-1* adrenoceptors down-regulate in rats following pharmacological treatments that elevate synaptic norepinephrine (Sulser et al. 1984; Ordway et al. 1991). Elevated concentrations of norepinephrine have been detected postmortem in limbic forebrain (Farley et al. 1978; Bird et al. 1979; Hornykiewicz 1986) in schizophrenic subjects as compared to control subjects. An elevation of norepinephrine in the cerebrospinal fluid of schizophrenics compared to controls has also been reported (Gomes et al. 1980; Lake et al. 1980; Sternberg et al. 1981; Van Kammen et al. 1989). Interestingly, measures of noradrenergic activity seem to be good indicators and/or predictors of the state of disease and its outcome (Hornykiewicz 1982, 1986; Van Kammen and Kelley 1991).

A reduction in *beta-1* adrenoceptor binding secondary to neuronal cell loss is possible given numerous postmortem and neuroimaging studies revealing hippocampal atrophy in schizophrenic subjects (Bogerts et al. 1985; Falkai and Bogerts 1986; Falkai et al. 1988; Benes et al. 1991). Cell loss (Falkai and Bogerts 1986) and/or reduced neuronal sizes (Benes et al. 1991) in the

hippocampus of schizophrenics has also been reported. However, reduced density of *beta-1* adrenoceptors simply attributable to cell loss seems unlikely, because binding to *alpha-2* adrenoceptors, another predominately postsynaptic noradrenergic receptor (see Ordway 1995), was not different in the same hippocampi of schizophrenics compared to control subjects. In fact, there was a tendency for higher, rather than lower, [^{125}I]PIC binding to *alpha-2* adrenoceptors in several hippocampal fields.

A malfunction of two brain mechanisms controlled by norepinephrine could contribute to psychotic behavior. One of those could be a defect in the noradrenergically controlled attentional (stimulus filtering) mechanisms of the locus coeruleus noradrenergic dorsal bundle that innervates the limbic forebrain area and the hippocampus. The second mechanism could be an alteration in the noradrenergic control of brain dopaminergic systems that generate motivational responses (see Hornykiewicz 1986). Our data support the hypothesis of noradrenergic overactivity in schizophrenia that leads to a down-regulation of *beta-1* adrenoceptors in the hippocampus, and indicate that alterations in central noradrenergic transmission may contribute to the neurochemical pathology of schizophrenia.

CONCLUSIONS

There is compelling evidence of a biological abnormality of central noradrenergic neurons in major depression and suicide, characterized by elevated levels of tyrosine hydroxylase (Zhu et al. 1995), elevated radioligand binding to *alpha-2* adrenoceptors (Ordway et al. 1994b), and reduced density of norepinephrine transporters (Klimek et al. 1997) in the noradrenergic locus coeruleus. Reduced levels of norepinephrine transporters, up-regulation of tyrosine hydroxylase, and up-regulation of *alpha-2* adrenoceptors are neurobiological changes observed after depletion of norepinephrine in the rat (Bylund and Martinez 1980; Lee et al. 1983; Bylund 1988; Melia et al. 1992). Therefore, postmortem findings suggest that major depression is characterized, at least in part, by a reduced availability of norepinephrine at the synapse. We would expect that noradrenergic receptors in projection areas of the locus coeruleus (e.g., hippocampus) would also be up-regulated in major depression or suicide. However, no significant differences in binding to *alpha-2*, *beta-1*, or *beta-2* adrenoceptors were observed in the frontal cortex and/or hippocampus from major depressives, suggesting that alterations in noradrenergic neurochemistry in depression are neuroanatomically specific. Previous studies demonstrating alterations in protein levels in noradrenergic cells of origin may reflect neurochemical changes in subpopulations of cells having discrete projections. The present findings do not exclude the possibility that *alpha-2*, *beta-1*, or *beta-2* adrenoceptors could be functionally abnormal; thatis, changes in second messenger systems or the affinity of norepinephrine for these receptors may be altered in depression. The demonstration of reduced *beta-1* adrenoceptor binding in the hippocampus of schizophrenic subjects relative to normal control and major depressive subjects provides further evidence for a role of norepinephrine in the biology of schizophrenia and distinguishes noradrenergic pathology in schizophrenia from that in major depression.

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