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Postmortem μ-opioid receptor binding in suicide victims and controls

G. Zalsman[1,2], **A. Molcho**[1,2], **Y. Huang**[1], **A. Dwork**[1], **S. Li**[1], and **J. J. Mann**[1]

[1] Division of Neuroscience, Department of Psychiatry, Columbia University, New York, NY, USA
[2] Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

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Summary. *Background*: Endogenous opiates may reinforce self-injurious behavior in animal and human subjects. Higher postmortem μ-opioid receptor binding is reported in some brain regions in young compared with older suicide victims. The present study compared opioid receptor binding kinetics in postmortem brains of young suicide victims and matched controls in two brain areas. *Methods*: The density (Bmax) and affinity (K_D) of the μ-opioid receptors were assayed postmortem using [^{3}H] DAGO in prefrontal cortex (PFC) and pre-post central gyri (PPCG) in 9 suicide victims and 10 controls, matched for age and gender ratio. *Results*: Binding indices did not differ between suicide victims and controls in either brain area and did not correlate with age. PFC Bmax was higher than PPCG Bmax ($F = 8.030$; $df = 1,16$; $p = .012$) for the combined sample. There was no brain region difference in K_D between PFC and PPCG, but the interaction between K_D and group was significant ($F = 5.890$; $df = 1,16$; $p = .027$). The K_D in the suicide victims was lower than controls in the PFC and higher than controls in the PPCG. *Conclusion*: Our study demonstrated more μ-opioid receptors in PFC compared with PPCG binding regardless of suicide status. The region-dependent differences in binding affinity in suicide victims may reflect regionally different opiate transmission.

Keywords: Postmortem, opioid receptors, suicide, prefrontal cortex, receptor binding.

Introduction

The possibility that endogenous opiates may reinforce self-injurious behavior has become a subject of renewed interest in studies of both human and animal models (Sandman et al., 2000; Shishido et al., 2000; Vaccarino and Kastin, 2001; White and Schultz, 2000). Naltrexone may decrease these behaviors in

human subjects (Sandman et al., 2000; White and Schultz, 2000). Self injurious behavior is more common during depression, and both human and animal studies report a modulatory role for β-endrophin in mood disorders (Yadid et al., 2000) and their treatment (Nakata et al., 1985).

μ-opioid receptor binding is reported to be higher in some brain regions of suicide victims (Gabilondo et al., 1995; Gross-Isseroff et al., 1990). Gross-Isseroff et al. (1990) demonstrated up to 9-fold greater μ-receptor binding in young (less than 41 years old) but not in old suicide victims' brains. This effect was significant in the pre and post central gyri (PPCG) and other fronto-parietal areas, but not in the PFC. Greater binding was due to more receptors (Bmax) and not higher affinity (K_D). Gabilondo et al. (1995) found 36–39% higher binding in the frontal cortex and caudate but not in the thalamus of suicide victims. K_D was not different. They also found that Bmax in PFC cortex and thalamus of suicide victims was positively correlated with age.

The present postmortem study examined the binding kinetics of μ-opiate receptors in two brain regions in a group of relatively young suicide victims and controls, matched for age and gender ratio, in order to re-assess the above findings. The PFC was selected, as it is a candidate brain region in the neurobiology of suicide (Mann, 2003), and the PPCG (primary motor and sensory areas) was selected as a control region.

Material and methods

Subjects

Samples from 19 postmortem brains in the brain bank of the New York State Psychiatric Institute, New York, were included in this study. Exclusion criteria were the presence of neuropathological changes and a direct evidence of drug abuse. Nine were suicides and ten had died suddenly from other causes, i.e. murder ($n = 8$) and motor vehicle accidents (MVA, $n = 2$). The methods of suicide were: hanging ($n = 4$), jumping from a height ($n = 3$), asphyxia ($n = 1$) and overdose ($n = 1$). The two groups were matched for age and gender (Table 1). The brain samples were frozen at $-80°C$ until assay. The range of postmortem delay was 10 to 15 hours. The institutional review board approved the study.

Dissection

Gray matter samples were dissected from right dorsal lateral prefrontal cortex (PFC) and pre-post central gyri (PPCG). Tissue was homogenized in 10 volumes of ice-cold 50 mM Tris-HCl buffer

Table 1. Demographic data and μ-opioid receptor binding indices of suicide victims and control subjects

	Brain area	Suicides	Controls	All sample	ANOVA
Age (yr)		22.8 ± 11.0	19.8 ± 2.9	21.2 ± 7.8	NS
Gender (M/F)		8M/1F	9M/1F	17M/2F	NS
Bmax (fmol/mg)	PFC	35.69 ± 14.05	33.96 ± 12.98	34.7 ± 13.08	$p = .012$[a]
	PPCG	23.71 ± 16.02	21.16 ± 12.71	22.37 ± 14.02	
K_D (nM)	PFC	$.69 \pm .20$	$.84 \pm .38$	$.77 \pm .31$	$p = .027$[b]
	PPCG	$.95 \pm .50$	$.68 \pm .39$	$.81 \pm .45$	

PFC Prefrontal cortex; *PPCG* Pre-post central gyrus. [a] ANOVA with repeated measure of PFC Bmax vs. PPCG Bmax; [b] interaction between K_D and group

(pH 7.4) using a polytron homogenizer, and centrifuged at 20,000 × g for 20 min (Sorvall, SS-34 rotor). The pellets obtained were again homogenized and centrifuged at 20,000 × g for 20 min. The final pellets were suspended in ice-cold 50 mM Tris-HCl buffer. All steps were performed at 4°C.

Binding assay

Membrane fractions (300 µg protein of suspended tissue) were incubated with different concentrations (0.1–4 nM) of [^{3}H] [D-Ala^2, MePhe^4, Gly-ol^5] enkephalin or ([^{3}H] DAGO), in a total volume of 500 µl. After an incubation for 60 min at 25°C in a shaking water bath, the reaction was terminated by rapid filtration (Cell Harvester, Brandel, Gaithersburg, MD, U.S.A.) through Whatman GF/B filters presoaked in 1 mg/ml bovine serum albumin for 60 min. Radioactivity was counted and nonspecific binding was determined in the presence of 10 µM naloxone. All assays were done in duplicate. The experiments were analyzed by using the LIGAND program. In one case, the frontal binding assay failed for technical reasons and it was excluded from the repeated measure analysis.

Statistical analysis

We used SPSS statistical software, edition 11.5.0 for Windows (2002) by SPSS Inc, Chicago IL. Student's T-test was employed to analyze differences in Bmax and K_D. Analysis of Variance (ANOVA) with repeated measures was employed to analyze group differences in binding indices (Bmax and Kd) across brain regions. All tests were two-tailed. The ± sign represents standard deviations.

Results

Demographic data and binding indices are summarized in Table 1. The age and male/female ratio did not differ between suicides and controls.

The Bmax in either PFC or PPCG did not differ between suicides and controls. However, higher Bmax was found in PFC compared to PPCG (F = 8.030,

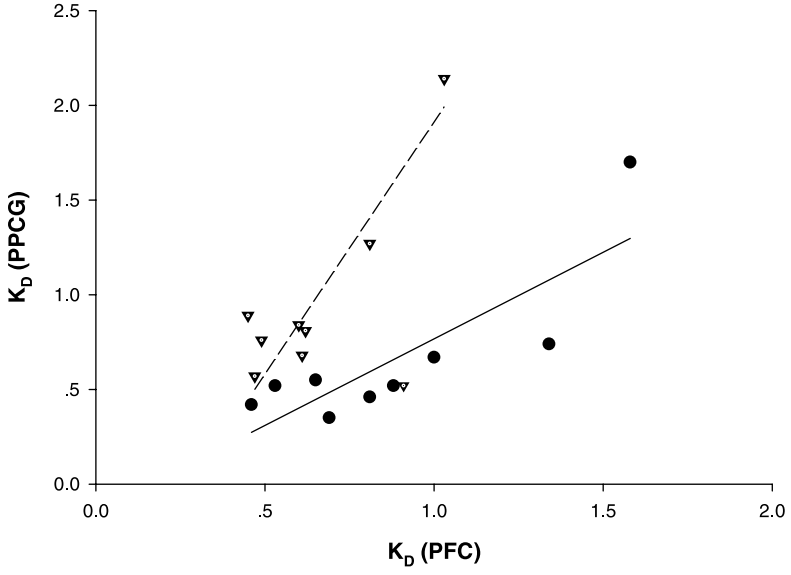

Fig. 1. Correlation between µ-opioid receptor affinity K_D (nM) in prefrontal cortex and pre-post central gyri in brains of suicide victims and controls. ▿ Suicide, ● Nonsuicide. Interaction between K_D and group: F = 5.890; df = 1,16; p = .027. *PFC* Prefrontal Cortex, *PPCG* Pre-Post Central Gyri

df = 1,16; p = .012). No group effect was observed (F = 0.407, df = 1,16; p = .533), on Bmax and the interaction between group and Bmax was not significant (F = .155, df = 1,16; p = .698).

K_D did not differ between the two brain-areas (F = .340; df = 1,16; p = .568), but the interaction between K_D and group was significant (F = 5.890; df = 1,16; p = .027). The K_D in the suicide victims was lower than controls in the PFC and higher than controls in the PPCG (see Table 1 and Fig. 1).

Age did not correlate with binding indices in the suicides: PPCG Bmax (r = .09, p = .817), PFC B_{max} (r = −.27, p = .508), PFC K_D (r = .06, p = .884) or PPCG K_D (r = .08, p = .836). There was also no correlation of binding indices with age in the control group or the combined sample in both brain regions (data not shown). The K_D in the PFC and PPCG were positively correlated in the whole sample (r = .51, p = .030) and on post-hoc testing within the control subjects (r = .69, p = .025) and at a trend level of significance in the suicide group (r = .67, p = .065) (Fig. 1).

Discussion

This study demonstrated higher postmortem μ-opioid receptor density (Bmax) in PFC compared with PPCG areas of human brain, regardless of the cause of death (i.e. suicide or other causes). No suicide effect was observed on Bmax in either of the two brain regions studied. Gabilondo et al. (1995) found 36–39% more prefrontal (Brodmann area 9, head of caudate and thalamus) μ-opioid receptors in suicide victims compared with controls (61 ± 7 fmol/mg vs. 44 ± 4, respectively, $p < .05$). In our sample, the mean Bmax was lower than their study, perhaps reflecting assay differences, or effects of postmortem delay (10–15 hours in our study; 17–46 hours in the Gabilondo study; 19–27 hours in the Gross-Isseroff study), or brain region differences in lipids concentration in the slides (Leslie et al., 1988). More likely, the different results in the three studies may be due to the use of a different drug for defining the non-specific binding in each of these studies (i.e. naltrexone 10 mM, ethropine 1 μM, and morphine 20 μM). We used the naltrexone (an antagonist) because it is a more specific ligand for the μ-opioid receptors (Cooper et al., 2003) and that may explain why we found a lower Bmax. Perhaps other non-μ-opioid receptor changes explain the findings in previous studies.

Higher binding in young suicide victims was reported by another group in the PPCG, which is a primary motor and sensory area (Gross-Isseroff et al., 1990). They did not find a significant difference in the PFC, which is a brain region thought to be associated with suicidality, and they did not correct for multiple testing of numerous brain areas. They reported Bmax that ranges from 189 ± 42 to 247 ± 35 fmol/mg in the prefrontal cortex of young suicide victims and 162 ± 33 to 206 ± 29 in the control group, however these differences were not statistically significant. In both pre-central and post-central gyri they found a significant difference ($p < .05$) in Bmax of young suicide victims (133 ± 46 and 233 ± 70, respectively) vs. controls (16 ± 10 and 26 ± 8, respectively) (Gross-Isseroff et al., 1990). Interestingly, the Bmax measures of both suicides and controls in our study are similar to their controls (23.71 ± 16.02 in suicides and 21.16 ± 12.71

in controls). That would suggest that the differences in results have something to do with the suicide sample studied. Gross-Isseroff et al. (1990) reported also that the Bmax of the opioid receptor correlated positively with age in most regions of the brain. Gabilondo et al. (1995) also reported a positive correlation of Bmax with age ($r = 74$, $p = .002$), however, unlike Gross-Isseroff et al. (1990), in suicide victims the relationship between age and Bmax could be found in the frontal area only. We did not observe such a correlation in our sample, which consisted primarily of young adults, neither in the suicides nor in the controls.

We found an interaction of brain region and group on K_D, such that suicides had higher affinity receptors in the PFC and lower affinity receptors in the PPCG. This could reflect differences in affinity or competition from endogenous opiates. Gross-Isseroff et al. (1990) did not find a significant difference in K_D between controls and suicide victims ($0.8\,nM \pm .19$ vs. $.53\,nM \pm .19$, respectively; superior frontal cortex) nor did Gabilondo et al. (1995) ($1.63\,nM \pm .34$ vs. $1.45\,nM \pm .25$, respectively, frontal cortex). However, the two papers find a direction of difference, namely higher affinity in the suicide group in PFC, to be the same as the effect we report, although Gabilondo et al. (1995) report a higher K_D than we report ($.84 \pm .38$ for controls and $.69 \pm .20$ for suicides), perhaps suggesting other receptors are also being assayed. Our use of a preincubation period may eliminate endogenous opiate transmitter that could compete with the ligand and suggest a higher K_D. A higher K_D could reflect super-sensitivity in response to lower endogenous opiate neurotransmitter levels.

Limitations

As in many other postmortem studies, one limitation is the lack of reliable information on the mental status and diagnosis of both suicides and controls close to the time of death. Therefore, it is possible that our results are related to mental disorders such as a mood disorder rather than suicide. Another limitation of postmortem studies is the small sample size, however, repeated measures ANOVA generally reduce the error terms and enhance the power of the analysis, resulting in the need for fewer subjects (Munro, 2001).

Exogenous opiates can affect binding results but were ruled out by toxicological analyses of body fluids. The same applies to other drugs of abuse and psychotropic medications. We cannot rule out enduring effects on receptors.

Our study identified differences in apparent affinity of opiate receptors in suicide victims that may reflect different levels of endogenous opiates in response to stress effects associated with the psychic pain that leads to suicide. However, in washed membranes, much of the endogenous peptides are metabolized or washed away and should not affect the binding. Zubieta et al. (2001) have demonstrated a significant reduction in regional receptor availability (binding potential was defined as $Bmax/K_D$) in the brains of healthy subjects undergoing sustained pain versus placebo, using positron emission tomography. They interpret their findings as reflecting opiate release (Zubieta et al., 2001). Our finding of a lower K_D (higher affinity) in the prefrontal cortex of suicide victims is consistent with a homeostatic response to lower endogenous opiate levels that has been reported in a group of patients with chronic self-injurious behaviors

(Sandman et al., 2000; White and Schultz, 2000), an effect that may also enhance the severity of sustained, severe mental pain. Altered μ-opioid receptor structure, as manifested by different K_D (Cooper et al., 2003), may also be associated with the higher threshold and pain tolerance that was observed in suicide attempters when compared to control subjects (Orbach et al., 1997).

Future studies can test these hypotheses by determining the relationship between endogenous opiate levels and receptor number and apparent binding affinity in serious suicide attempters.

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Authors' address: J. J. Mann, M.D., Division of Neuroscience, Department of Psychiatry, Columbia University, 1051 Riverside Drive, Box 42, New York, NY 10032, USA, e-mail: jjm@columbia.edu