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Platelet serotonin concentration and suicidal behavior in combat related posttraumatic stress disorder

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Abstract

Posttraumatic stress disorder (PTSD) is a serious and global problem, a psychiatric disorder that frequently occurs with different comorbidities, and is associated with a high suicide rate. Pathophysiologically, both PTSD and suicidal behavior are related to disturbances in the central serotonergic system. Serotonin (5-hydroxytryptamine, 5-HT) controls emotional behavior, anxiety, impulsivity and aggression, and nearly all known antidepressants and antianxiety drugs affect 5-HT transmission. Platelet 5-HT can be used as a limited peripheral marker of the central serotonergic synaptosomes, since it is related to particular basic psychopathological characteristics of several psychiatric disorders. Platelet 5-HT concentration has been reported to be similar in PTSD subjects and healthy controls, but suicidal patients across different psychiatric diagnoses have reduced platelet 5-HT concentration. This study examined platelet 5-HT concentration by the spectrofluorimetric method in male subjects: 73 suicidal and 47 non-suicidal veterans with current and chronic combat related PTSD, 45 suicidal and 30 non-suicidal comparative non-PTSD subjects and 147 healthy men. The presence of suicidal behavior (score=0, non-suicidal; scores ≥ 1, suicidal) was assessed with the Hamilton Depression Rating Scale-17 (HDRS). Platelet 5-HT concentration was significantly lower in suicidal PTSD and non-PTSD patients compared to non-suicidal patients or healthy controls. Since the majority of patients scored very low on item 3 of HDRS, no significant correlation between suicidal scores and platelet 5-HT concentration was found. These results show that reduced platelet 5-HT concentration is related to suicidal behavior in PTSD, and suggest that platelet 5-HT concentration might be used as a peripheral marker to predict suicidal behavior across psychiatric diagnoses.

Keywords: biological marker; male subjects; platelet serotonin; combat related posttraumatic stress disorder; suicidal behavior

Abbreviations: ANOVA, analysis of variance; CAPS, Clinician Administered PTSD Scale; HAMA, Hamilton Rating Scale for Anxiety; HDRS, Hamilton's Rating Scale for Depression 17-item; HVA, homovanillic acid; 5-HIAA, 5-hydroxylindoleacetic acid; 5-HT, 5-hydroxytryptamine, serotonin; MAO, monoamine oxidase; PANSS, Positive and Negative Syndrome Scale; PRP Platelet rich plasma; PTSD, posttraumatic stress disorder; SSRI, selective serotonin reuptake inhibitors; SCID, Structured Clinical Interview.

Introduction

Posttraumatic stress disorder (PTSD) is a serious and global problem, a psychiatric disorder that frequently occurs with different comorbidities, and is associated with a high suicide rate (Kessler et al., 1999; Kozaric-Kovacic and Kocijan-Hercigonja 2001). The neurobiology of PTSD includes alterations in neuroendocrine and neurotransmitter systems and morphological changes such as reduced hippocampal volume (Ballenger et al., 2004; Van Praag, 2004; Nemeroff et al., 2006). The most frequent finding is altered function of the hypothalamic-pituitary-adrenal axis, with hypersecretion of corticotrophin-releasing factor, with paradoxically low to normal cortisol levels, and a super-suppression of cortisol secretion after a low-dose of dexamethasone in a dexamethasone suppression test. The disrupted neurotransmitter systems in PTSD are norepinephrine, serotonin (5-hydroxytryptamine, 5-HT), glutamate, γ-aminobutyric acid, and endogenous opioids. Combat related PTSD is accompanied with a high risk of suicide, and military experience, guilt about combat actions, survivor guilt, depression, anxiety and severe PTSD are significantly associated with suicide attempts (Freeman et al., 2000; Ferrada-Noli et al., 1998; Price et al., 2004).

Suicidal behavior is a major social and public health problem which significantly increases the death toll worldwide. The complex genetic, neurobiological, and psychosocial origins of suicidal behavior are still unclear, but abnormalities in the serotonergic system have been found in suicide victims (Creminter et al., 1994; Mann, 2002; Mann, 2003). These findings are in line with the impairments of the central 5-HT neurotransmission found in a variety of behavioral and physiological abnormalities, psychiatric disorders, and in extreme violence, hostility, impulsivity and aggression (Lucki, 1998; Stahl, 1998). Suicide risk has been associated with alterations in serotonergic correlates such as lower 5-hydroxylindoleacetic acid (5-HIAA) and homovanillic acid (HVA) levels in patients with a history of suicide attempts (Jones et al., 1990; Nordstron et al., 1994), lower ^{3}H-imipramine binding and 5-HT uptake in platelets of suicide attempters, and lower central 5-HT function (Roy and Linnoila 1988; Mann 1998).

Blood platelets can be used as limited peripheral model for central 5-HT neuron function (Stahl, 1985; Andres et al., 1993; Hrdina, 1994; Camacho and Dimsdale, 2000; Mendelson, 2000; Goveas et al., 2004), since platelets share some similarities with the central 5-HT synaptosomes. The similarities lay in the pharmacodynamics of 5-HT, including uptake, storage and release of 5-HT, platelet monoamine oxidase (MAO) type B, 5-HT transporters

(Lesch et al., 1994), 5-HT2 receptors (Andres et al., 1993), α_2-adrenergic receptors, and binding sites for ^{3}H-imipramine, ^{3}H-paroxetine, and ^{3}H-LSD, and signaling mechanism which share identical pharmacologic and kinetic characteristics with the central nervous receptors and binding sites. The differences between blood platelets and 5-HT neurons are in their function, since platelets lack a nucleus, and they do not synthesize 5-HT (Hrdina, 1994). Peripheral serotonergic markers, such as platelet 5-HT, have been associated with particular basic psychopathological characteristics, i.e. trait markers (Askenazy et al., 2000; Mueller-Oerlinghausen et al., 2004). Although the significance of platelet 5-HT as a marker of suicidality has been recently questioned (Mueller-Oerlinghausen et al., 2004), lower platelet 5-HT has been associated with suicidal behavior in psychotic and non-psychotic depressed patients (Muck-Seler et al., 1996; Pivac et al., 1997), and in patients with first episode of psychosis (Marcinko et al., 2007).

To our knowledge, there are no studies that have examined the association of platelet 5-HT with suicidal behavior in veterans with combat related PTSD. Hence, based on our previous data (Muck-Seler et al., 1996; Pivac et al., 1997; Marcinko et al., 2007), the hypothesis of this study was that platelet 5-HT concentration would be reduced in suicidal patients compared to non-suicidal PTSD patients. The aim of the study was to determine the differences in platelet 5-HT concentration among male suicidal and non-suicidal veterans with combat related PTSD, male psychiatric controls with diagnoses other than PTSD (non-PTSD patients), and healthy men, and to determine whether platelet 5-HT concentration might be used as a peripheral platelet marker to predict the risk of suicidal behavior.

2. Methods

2.1. Subjects and clinical evaluation

The study included male medication-free participants. The patients (120 male war veterans with combat related PTSD), had current and chronic PTSD, diagnosed using the Structured Clinical Interview for DSM-IV (SCID, First et al., 2000) based on DSM-IV Disorders (APA, 1994), the Clinician Administered PTSD Scale (CAPS, Weathers et al., 2001), the Hamilton Rating Scale for Depression-17 (HDRS, Hamilton, 1960) and Anxiety (HAMA, Hamilton, 1959), and the Positive and Negative Syndrome Scale (PANSS, Kay et al., 1987). The patients were recruited from October 2001- March 2005 from the Referral Centre for the Stress-related Disorders of the University Hospital Dubrava in Zagreb, Croatia.

The assessments and ratings were conducted by psychiatrists with extensive experience in stress-related disorders. Psychiatrists administered the SCID, CAPS, HDRS, HAMA, PANSS, as well as an additional psychiatric interview, which is routinely performed in every day clinical pratice at the department. The PANSS was used to detect pychotic features in PTSD patients (Kozaric-Kovacic and Pivac, 2006). Suicidal behavior was assessed by psychiatrists using item 3 from the HDRS 17-item scale (Hamilton, 1960): score 0, which indicates absence of any suicidal thoughts or ideation, score 1 (a person feels life is not worth living), score 2 (a person wishes he/she was dead or has any thoughts of possible death to him/herself), score 3 (a person has suicide ideas or gestures), and score 4 (a person attempted suicide). According to the item 3 of the HDRS, patients were categorized into two groups (Roggenbach et al., 2007): non-suicidal subjects (with 0 scores) and suicidal subjects (with scores ≥ 1). Among the PTSD patients, 73 male veterans were suicidal, while 47 male veterans were non-suicidal.

Inclusion criteria: medication-free male combat veterans between 18-65 years old. Exclusion criteria: a family history of psychosis or PTSD, a prior episode of any psychosis, a positive history of schizophrenia spectrum disorders, bipolar disorder, neurodegenerative disorders, history of cognitive dysfunction or mental retardation, alcohol dependence, past or current alcohol or other substance abuse within 3 months, serious concomitant medical condition, clinically significant abnormalities in electrocardiogram or laboratory findings, including positive urine screen for illicit drugs and alcohol.

Two control groups were established for this study. The first group consisted of non-PTSD comparison psychiatric patients: 75 male patients with diagnoses other than PTSD (depression (N=35), psychosis (N=7), acute stress disorder (N=12), personality disorders (N=12), other diagnoses (N=9)). All patients, diagnosed using the SCID, were categorized into suicidal and non-suicidal patients, according to the item 3 of the HDRS: 45 suicidal and 30 non-suicidal patients. These patients were drug-naïve or drug free for at least 2 weeks, and were not taking selective serotonin reuptake inhibitors (SSRI) during the previous 6 weeks. The second control group consisted of 147 medication-free healthy men with no personal or family history of psychopathology, no previous history of suicide attempt or family history of suicide, free of psychiatric disorders, and no current medical treatment.

Study participants in the PTSD in the non-PTSD comparison group were recruited as consecutive in- and out-patients admitted during the indicated recruitment period, among patients who fulfilled the inclusion and exclusion criteria for either group. Healthy controls were recruited consecutively among people attending the ward as part of their regular medical

check-ups, who fulfilled the inclusion and exclusion criteria for the healthy control group. Groups were matched on mean age, education, and drug status.

All participants completed the questionnaire regarding their medical history, drinking, and smoking habits. Patients and healthy controls were sampled in the same period of the year. Written informed consent was obtained from all participants, under procedures approved by the Ethics Committee of the University Hospital Dubrava, Zagreb, Croatia, and in accordance with the Declaration of Helsinki.

2.2. Blood sample collection and testing

Blood samples (8 ml) were drawn in a plastic syringe with 2 ml of acid citrate dextrose anticoagulant at 08.00 h. Platelet rich plasma (PRP) was obtained after centrifugation of whole blood at 950g for 30 sec at room temperature and platelets were sedimented by further centrifugation of PRP at 10,000g for 5 min in a refrigerated centrifuge. The platelet pellet was washed with saline and centrifuged again. The sedimented platelets were frozen and stored at -20°C no longer than 7 days before being assayed for 5HT concentration.

2.3. Platelet 5-HT measurement

Platelet 5-HT concentration was determined by the spectrofluorimetric method, as previously described (Pivac et al., 2001). Briefly, platelets were destroyed by sonication (20 kHz, amplitude 8 x 10^{-3} mm for 30 sec). Specimens of standard, blank (water) and platelet sonicates were analyzed in duplicate. All samples were deproteinized with 1 ml of 10% $ZnSO_4$ and 0.5 ml of 1 N NaOH. For the preparation of fluorophore, 0.2 ml of L-cysteine (0.1%) and 1.2 ml of orthophthalaldehyde (0.05%) were added to deproteinized samples. The measurement of the 5-HT fluorescence was performed on a Varian Spectrophotofluorimeter Cary Eclipse, on an exciting wavelength of 345 nm and emitted wavelength of 485 nm. Platelet protein concentrations were measured by the method of Lowry et al. (1951).

2.4. Statistical analysis

The statistical analysis of the data was conducted with Statistica, release 6, Statsoft (Tulsa, Oklahoma, USA), and with Sigmastat 2.0 (Jandell Scientific Corp. San Raphael, California, USA). Results are shown as means ± standard deviations (SD) with 95% confidence intervals (CI). The statistical evaluation of the results was performed using one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test. The correlation between

parameters was determined by a Pearson's coefficient of correlation. All results were interpreted at 5% significance level (α=0.050).

3. Results

3.1. Characteristics of the subjects

Data on the age and clinical scales scores in the veterans with PTSD and in non-PTSD patients are summarized in Table 1. There were no significant (F=2.556; df=3,191; p=0.057, *ANOVA*) differences in age between patients. Total CAPS scores, determined in PTSD patients, were similar (F=1.353; df=1,118; p=0.248) between suicidal and non-suicidal PTSD patients.

All patients were evaluated with HDRS, and total HDRS scores differed significantly between the patient groups (F=31.569; df=3,191; p<0.001). Non-suicidal non-PTSD patients had significantly lower total HDRS scores than all other groups: suicidal non-PTSD patients (p<0.002), non-suicidal PTSD patients (p<0.001), and suicidal PTSD patients (p<0.001). Suicidal and non-suicidal PTSD patients had similar (p= 0.261) total HDRS scores, Table 1.

Regarding suicide scores on HDRS item 3, PTSD patients had significantly higher suicide scores than non-PTSD patients (F=9.246; df=1,116; p<0.003).

The total HAMA scores differed significantly between patients (F= 12.474; df=3,191; p<0.001), since suicidal PTSD patients had significantly higher (p<0.001) HAMA scores than non-suicidal PTSD patients, and suicidal non-PTSD patients had significantly higher (p<0.002) HAMA scores than non-suicidal non-PTSD patients.

The total PANSS scores also differed significantly between patients (F=14.161; df=3,191; p<0.001), with suicidal PTSD (p<0.036) and non-PTSD (p<0.001) patients having significantly higher total PANSS scores than the corresponding non-suicidal patients. Non-suicidal PTSD patients had significantly (p<0.005) higher total PANSS scores than non-suicidal non-PTSD patients, Table 1.

Among PTSD patients, 73 (61%) were suicidal, of those 32 (44%) felt that life is not worth living; 18 (25%) wished that they were dead or had thoughts of possible death; 15 (20%) had suicide ideas or gestures; and 8 (11%) had previous suicide attempts.

Of the 45 (67%) suicidal comparison patients who had diagnoses other than PTSD (non-PTSD patients), 33 (73%) felt that life is not worth living; 7 (16%) wished that they were dead or had thoughts of possible death; 5 (11%) had suicide ideas or gestures; and none had attempted suicide.

3.2. Platelet 5-HT concentration between suicidal and non-suicidal PTSD patients, psychiatric non-PTSD controls, and healthy control subjects

Platelet 5-HT concentration differed significantly between all groups (*F=5.546; df=4,337; p*<0.001*, ANOVA*), Fig. 1. *Tukey's test* revealed significantly lower platelet 5-HT concentration in suicidal compared to non-suicidal (*p*<0.029) PTSD patients or healthy controls (*p*<0.001). Significantly higher platelet 5-HT concentration was found in non-suicidal compared to suicidal psychiatric comparison controls (*p<0.020*) or healthy controls (*p*<0.013). Platelet 5-HT concentration did not differ significantly between healthy control subjects and non-suicidal PTSD patients (*p*=0.712*).* Suicidal non-PTSD patients had marginally lower platelet 5-HT concentration than healthy control subjects (*p*=0.072*).*

3.3. The lack of effect of smoking on platelet 5-HT in patients

Platelet 5-HT concentration did not differ significantly between PTSD and non-PTSD patients (*F=0.808; df=3,151; p*=0.493**,** *ANOVA*), who were categorized according to the smoking status into smokers or nonsmokers (Fig. 2).

3.4. Platelet 5-HT concentration and severity of suicidal behavior in suicidal patients

There were no significant correlations between platelet 5-HT and HDRS scores for suicidal behavior (item 3 of the HDRS) in suicidal PTSD patients (*r*=0.085; *p*=0.508), or suicidal non-PTSD patients (*r*=-0.148; *p*=0.354).

4. Discussion

4.1. Principal findings

In this comparative study with male subjects, suicidal veterans with combat related PTSD, and comparative suicidal patients with diagnoses other that PTSD (depression, psychosis, acute stress disorder, personality disorders, and other diagnoses), had lower platelet 5-HT

concentration than non-suicidal patients, while suicidal PTSD patients had lower platelet 5-HT concentration than control subjects. These findings are consistent with significantly decreased platelet 5-HT concentration found in suicidal depressed patients (Mann et al., 1992; Muck-Seler et al., 1996; Pivac et al., 1997; Rogenbach et al., 2007), in patients with a history of violent suicide attempts (Alvarez et al., 1999), in suicidal patients with a first episode of psychosis (Marcinko et al., 2007), in suicidal patients with schizophrenia (Braunig et al. 1989), or schizoaffective disorder (Rao et al. 1994). Athough the importance of platelet 5-HT as a biological marker of suicidal behavior has recently been disputed (Mueller-Oerlinghausen et al., 2004), the present results, obtained on a large sample of male PTSD patients and male comparison patients without PTSD, together with previous data, obtained on a large number of psychotic and non-psychotic male and female depressed patients (Muck-Seler et al., 1996; Pivac et al., 1997), and a smaller number of male patients in acute psychosis (Marcinko et al., 2007), argue that reduced platelet 5-HT concentration might serve as a predictor of suicidal behavior. In line with our data, attempted or completed suicides were reported to be related to reduced levels of 5-HT and 5-HIAA in blood and cerebrospinal fluid (CSF), (Nordstrom et al., 1994). Furthermore, suicide attempts were associated with lower CSF 5-HIAA concentrations in depressed patients (Mann and Malone, 1997), with fewer prefrontal cortex 5-HT transporter binding sites (Mann et al., 2003), and upregulation of serotonergic post-synaptic 5-HT1A and 5-HT2A receptors in the brain (Arora and Meltzer, 1989). In addition, more 5-HT2A binding and more mRNA in the postmortem prefrontal cortex tissue of suicide victims was found (Pandey et al., 2002).

In a review questioning the use of platelet markers as predictors of suicidal behavior (Mueller-Oerlinghausen et al., 2004), the authors stressed the importance of large sample sizes and matching the patients for age, gender, season, drug status, smoking habits and different comorbidities. In our previous studies (Muck-Seler et al., 1996; Pivac et al., 1997) we carefully matched patients for these confounding factors. Namely, gender differences were detected in platelet 5-HT concentration in previous studies (Oxenkrug, 1979; Muck-Seler et al., 1996; 1999; Pivac et al., 2001; Roggenbach et al., 2007), and thereafter in our previous studies measuring platelet 5-HT all our patients were divided according to the sex. The present study included only male subjects, and therefore gender matching was not necessary. Smoking does not affect platelet 5-HT concentration in healthy subjects (Pivac et al., 2004) or in patients (present study), hence the data from smokers and nonsmokers were collapsed. Alcohol abuse can lower platelet 5-HT concentration (Pivac et al., 2004), and therefore we

excluded patients with alcohol dependence, past or current alcohol or other substance abuse within 3 months. Although seasonality does not have any significant impact on platelet 5-HT concentration (Jakovljevic et al., 1997; Pivac et al., 2003), patients and comparative subjects were sampled consecutively in the same recruitment period. Medication such as SSRI reduces platelet 5-HT concentration (Muck-Seler et al., 2002; Muck-Seler et al 2005; Pivac et al., 2003), and to avoid this influence, the participants in this study were not prescribed SSRI in previous 6 weeks. Although platelet 5-HT does not appear to differ according to diurnal rhythm (unpublished data), all subjects were sampled at the same time of day (i.e. at 08.00 a.m.). Our study consisted of a large number of male subjects (120 war veterans, with current and chronic PTSD, and 75 patients with diagnoses other than PTSD, as psychiatric control subjects; categorized according to suicidal behavior) and 147 medication-free healthy control subjects. It has been reported that other comorbid psychiatric diagnoses such as depression, schizophrenia, or borderline personality disorder, might affect biological parameters associated with suicidal behavior (Mueller-Oerlinghausen et al., 2004). In contrast to this suggestion, in veterans with PTSD, comorbid depression did not significantly affect platelet 5-HT concentration (Muck-Seler et al., 2003). Since our PTSD patients did not suffer from schizophrenia or borderline personality disorder, and only 2 patients had comorbid depression, we can exclude the possibility that these diagnoses significantly affected platelet 5-HT concentration. In addition, in our study suicidal comparison non-PTSD patients, with diagnoses of depression, psychosis, acute stress disorder, and personality disorders, also had lower platelet 5-HT concentration than non-suicidal patients, indicating a reduced platelet 5-HT concentration associated with suicidal behavior across different diagnoses. Therefore, although the usefulness of platelet 5-HT, MAO-B, 5-HT2A receptor binding and tryptophan availability as potential biological markers for suicidal behavior has been questioned (Mueller-Oerlinghausen et al., 2004; Lauterbach et al., 2006), the same author group has recently reported significantly lower platelet 5-HT concentration in acute suicidal vs. non-suicidal depressed patients (Roggenbach et al., 2007). Hence, our data support the suggestion that low serotonergic function is related to suicidal ideation and other personality traits characteristic for suicidal behavior, possibly through the collapse of the normal restraint mechanisms, thus contributing to the development of suicidal behavior (Lauterbach et al., 2006). The lack of a significant correlation between suicide HDRS scores and platelet 5-HT concentration in veterans with PTSD and comparison non-PTSD patients was to be expected, since the majority of patients scored very low on item 3 of HDRS, and only few patients (7%) had attempted suicide.

If platelet 5-HT concentration had differed significantly between non-suicidal patients with PTSD and healthy control group, the significance of the altered platelet 5-HT concentration in suicidal behavior would be difficult to explain, since altered platelet 5-HT concentration might be related to the presence of psychotic symptoms such as delusions (Pivac et al., 2006a), severe appetite loss (Muck-Seler et al., 2003), or some other traits in PTSD patients. However, in the present and our previous studies (Muck-Seler et al., 2003; Pivac et al., 2002; Pivac et al., 2006a; Pivac et al., 2006b), as well as in work from other groups (Maguire et al., 1998; Mellman and Kumar, 1994; Cicin-Sajn et al., 2000), platelet 5-HT concentration was similar between PTSD patients and corresponding control subjects. These data confirm our hypothesis that the reduced platelet 5-HT concentration in suicidal veterans with combat related PTSD is a trait marker, predictive of suicidal behavior in PTSD. Therefore, our data agree with the presumption that biological parameters are more likely associated with single aspects of experience and behavior, such as suicidal behavior, than with a nosological constructs or entities (Mueller-Oerlinghausen et al., 2004; van Praag, 2004).

Serotonin regulates divergent behaviors (Stahl, 1998; Lucki 1998) including suicidal behavior (Roy and Linoilla, 1988; Mann et al., 1996, Mann, 2003), and serotonergic 5HT1A and 5HT2A receptors mediate anxiety (Bonne et al., 2004), antisocial and aggressive behavior (Goveas et al., 2004; Popova, 2006), impulsivity and violence (Askenazy et al., 2000). Suicidal behavior is associated with a deficient 5-HT system (Mann et al., 1998), and altered 5-HT functioning was found in the ventromedial and lateral prefrontal cortex, which might be associated with the predisposition to suicidal behavior due to the impaired restraint mechanisms (Oquendo et al., 2003; Lauterbach et al., 2006). Our findings of the lower platelet 5-HT concentration in suicidal PTSD and comparative psychiatric patients suggest that platelet 5-HT concentration is related to particular trait markers (Askenazy et al., 2000; Peirson et al., 1999), and that platelet 5-HT may distinguish between PTSD subjects with and without suicidal behavior.

A few limitations of the study should be mentioned: platelet 5-HT was assessed only in one time point, and suicidal behavior was determined using HDRS item 3 scores (Rogenbach et al., 2007). Accordingly, patients were divided into non-suicidal (0 scores) and suicidal patients (with 1-4 scores, i.e. with feelings that life is not worth living, with wishes of death or any thoughts of possible death; suicide ideas or gestures, or suicide attempts). We should

emphasize that the strength of our study was in a large number (195) of patients, including PTSD and comparison psychiatric patients, of which 128 patients (61%) were categorized as suicidal. Out of them, 55% felt life is not worth living, 21% wished he/she was dead or had any thoughts of possible death to him/herself, and only a few patients had suicide ideas or gestures (17%), or suicide attempts (7%), as can be seen from the average (1.32-1.84) suicide scores. Therefore, our results show more merit, since platelet 5-HT concentration was significantly lower in patients with less pronounced suicidal behavior.

Suicidal behavior is a major complication of psychiatric disorders, and according to the WHO, it accounts for almost 2% of the deaths in the world (Bondy et al., 2006). In Croatia, the suicide rate oscillated depending on the source of the data (Register of Suicide of the Ministry of Interior, and Croatian Bureau of Statistics) for the period between 1985-2000 (i.e. pre-war, war and post-war periods in Croatia) (Grubisic-Ilic et al., 2002; Kozaric-Kovacic et al., 2002a; Kozaric-Kovacic et al., 2002b). The average suicide rate in Croatia was 17.83 - 19.57 per 100 000 people in pre-war period, 16.59-19.52 during war and 17.78-19.78 in the post-war period (Grubisic-Ilic et al., 2002; Kozaric-Kovacic et al., 2002a; Kozaric-Kovacic et al., 2002b). However, a higher frequency of suicide was found in younger men during war, presumably due to the fact that a lot of veterans suffered from PTSD, but did not receive proper treatment during combat (Kozaric-Kovacic et al., 2002a). Therefore, an important goal in the treatment strategies of PTSD is to elucidate the association between biological biomarkers and suicidal behavior in PTSD, to facilitate the identification of the subjects who are prone to develop suicidal behavior, and to detect suicidal patients, in order to prevent suicidal attempts and to provide proper treatment for individual patients with PTSD at any given stage of the illness.

In conclusion, our results have shown that suicidal veterans with combat related PTSD and comparison psychiatric non-PTSD patients, had lower platelet 5-HT concentration than non-suicidal patients, indicating a reduced platelet 5-HT concentration associated with suicidal behavior. Our data suggest that platelet 5-HT might be used as an easily obtainable peripheral marker that might detect suicidal behaviour in combat related PTSD (present study), depression (Muck-Seler et al., 1996; Pivac et al., 1997), acute psychosis (Marcinko et al., 2007), i.e. that platelet 5-HT might differentiate between suicidal and non-suicidal behavior across different psychiatric diagnoses, after carefull matching the patients for age, gender, diagnoses, and drug status.

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REFERENCES

Alvarez JC, Cremniter D, Lesieur P, Gregoire A, Gilton A, Macquin-Mavier I (1999). Low blood cholesterol and low platelet serotonin levels in violent suicide attempters. Biol Psychiatry; 45: 1066-1069.

American Psychiatric Association (1994). Diagnostic and Statistical Manual of Mental Disorders. 4th ed. American Psychiatric Press, Washington, DC.

Andres AH, Rao MA, Ostrowitzki S, Enzian W (1993). Human brain cortex and platelet serotonin$_2$ receptor binding properties and their regulation by endogenous serotonin. Life Sci; 52: 313-321.

Arora RC, Meltzer HY (1989). Increased serotonin2(5-HT2) receptor binding as measured by 3H-lysergic acid diethylamide (3H-LSD) in the blood platelets of depressed patients. Life Sci; 44: 725–734.

Askenazy F, Caci H, Myquel M, Darcourt G, Lecrubier Y (2000). Relationship between impulsivity and platelet serotonin content in adolescents. Psychiatry Res; 94: 19-28.

Ballenger JC, Davidson JRT, Lecrubier Y, Nutt DJ, Marshall RD, Nemeroff CB, et al. (2004).

Consensus Statement Update on Posttraumatic Stress Disorder From the International Consensus Group on Depression and Anxiety. J Clin Psychiatry; 65 (suppl 1): 55–62.

Bondy B, Buettner A, Zill P (2006). Genetics of suicide. Mol Psychoiatry; 4: 1-16.

Bonne O, Grillon C, Vythilingam M, Neumeister A, Charney DS (2004). Adaptive and maladaptive psychobiological responses to severe psychological stress: implications for the discovery of novel pharmacotherapy. Neurosci Biobehav Rev; 28: 65–94.

Braunig P, Rao ML, Fimmers R. (1989). Blood serotonin levels in suicidal schizophrenic patients. Acta Psychiatr Scand; 79: 186-189.

Camacho A, Dimsdale JE (2000). Platelets and psychiatry: lessons learned from old and new studies. Psychosom Med; 62: 326-336.

Cicin-Sajn L, Mimica N, Hranilovic D, Balija M, Ljubin T, Makaric G, et al. (2000). Posttraumatic stress disorder and platelet serotonin measures. J Psychiatr Res; 34: 155–161.

Cremniter D, Thenault M, Jamain S, Meidinger A, Delmas C, Gaillard M (1994). Serotonin and suicide: a preliminary study concerning a sample of violent suicidal patients. Prog Neuropsychopharmacol Biol Psychiatry; 18: 871-878.

First M, Spitzer R, Williams J, Gibbon M (2000). Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I). In: Handbook of Psychiatric Measures, Washington, DC: American Psychiatric Association. p. 49-53.

Ferrada-Noli M, Asberg M, Ormstad K, Lundin T, Sundbom E (1998). Suicidal behavior after severe trauma, part 1: PTSD diagnoses, psychiatric comorbidity, and assessments of suicidal behavior. J Trauma Stress; 11: 103–112.

Freeman TW, Roca V, Moore WM (2000). A comparison of chronic combat-related (PTSD) patients with and without a history of suicide attempt. J Nerv Ment Dis 2000; 188: 460–463.

Goveas JS, Csernansky JG, Coccaro EF (2004). Platelet serotonin content correlates inversely with life history of aggression in personality-disordered subjects. Psychiatry Res; 126: 23-32.

Grubisic-Ilic M, Kozaric-Kovacic D, Grubisic F, Kovacic Z (2002)**.** Epidemiological study of suicide in the Republic of Croatia-comparison of war and post-war periods and areas directly and indirectly affected by war. Eur Psychiatry;17: 259-264.

Hamilton MC (1959). Hamilton anxiety scale (HAMA). In: Schutte NS, Malouff JM, editors. Sourcebook of adult assessment (Applied clinical psychology). NY: Plenum Press; p. 154-157.

Hamilton M (1960). A rating scale for depression. J Neurol Neurosurg Psychiatr; 23: 56-62.

Hrdina PD (1994). Platelet serotonergic markers in psychiatric disorders: use, abuse and limitations. J Psychiatry Neurosci; 19: 87-88.

Jakovljevic M, Muck-Seler D, Pivac N, Ljubicic D, Bujas M, Dodig G (1997). Seasonal influence on platelet 5-HT levels in patients with recurrent major depression and schizophrenia. Biol Psychiatry; 41: 1028-1034.

Jones JS, Stanley B, Mann JJ, Frances AJ, Guido JR, Traskman-Bendz L, et al. (1990). CSF 5-HIAA and HVA concentrations in elderly depressed patients who attempted suicide. Am J Psychiatry; 147: 1225–1227.

Kay SR, Fiszbein A, Opler LA (1987). The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophr Bull; 13: 261-276.

Kessler RC, Borges G, Walters EE (1999). Prevalence of and risk factors for lifetime suicide attempts in the National Comorbidity Survey. Arch Gen Psychiatry; 56: 617–626.

Kozaric-Kovacic D**,** Grubisic-Ilic M, Grubisic F, Kovacic Z (2002a). Suicide rates and methods of suicide before, during and after the war in Croatia (1985-2000). Natl Med J India; 15: 356-367.

Kozaric-Kovacic D, Grubisic-Ilic M, Grubisic F, Kovacic Z (2002b). Epidemiological indicators of suicides in the Republic of Croatia. Drus Istraz; 57:155-170.

Kozaric-Kovacic D, Kocijan-Hercigonja D (2001). Assessment of PTSD and Comorbidity. Mil Med; 41: 78-83.

Kozarić-Kovačić D, Pivac N (2006) Psychotic features of combat related chronic posttraumatic stress disorder and antipsychotic treatment. In: Roy, M, editor. Novel approaches to the diagnosis and treatment of posttraumatic stress disorder.. NATO Security through Science series E: Human and Societal Dynamics – Vol 6. Section 1: Epidemiology and pathophysiology of PTSD. Amsterdam: IOS Press; p. 42-55.

Lauterbach E, Brunner J, Hawellek B, Lewitzk U, Ising M, Bondy B, et al. (2006). Platelet 5-HT2A receptor binding and tryptophan availability in depression are not associated with recent history of suicide attempts but with personality traits characteristic for suicidal behavior. J Affect Disord; 91: 57– 62.

Lesch KP, Baling U, Gross J, Strauss K, Wolozin B, Murphy DL, et al. (1994). Organization of the human serotonin transporter gene. J Neural Transm; 95: 157-162.

Lowry OH, Rosenbrough NS, Farr AC, Randall RJ (1951). Protein measurement with the Folin phenol reagent. J Biol Chem; 193: 265-75.

Lucki I (1998). The spectrum of behaviors influenced by serotonin. Biol Psychiatry; 44: 151-162.

Maguire K, Norman T, Burrows G, Hopwood M, Morris P (1998). Platelet paroxetine binding in post-traumatic stress disorder. Psychiatry Res; 77: 1-7.

Mann JJ, McBride PA, Brown RP, Linnoila M, Leon AC, DeMeo M, et al. (1992). Relationship between central and peripheral serotonin indexes in depressed and suicidal psychiatric inpatients. Arch Gen Psychiatry; 49: 442–446.

Mann JJ, Henteleff RA, Lagattuta TF, Perper JA, Li S, Arango V (1996). Lower 3H-paroxetine binding in cerebral cortex of suicide victims is partly due to fewer high affinity, non-transporter sites. J Neural Transm; 103: 1337–1350.

Mann JJ, Malone KM (1997). Cerebrospinal fluid amines and higher lethality suicide attempts in depressed inpatients. Biol Psychiatry; 41: 162–171.

Mann JJ (1998). The neurobiology of suicide. Nat Med; 4: 25–30.

Mann JJ (2002). A current perspective of suicide and attempted suicide. Ann Intern Med; 136: 302–311.

Mann JJ (2003). Neurobiology of suicidal behaviour. Nat Rev Neurosci; 4: 819–828.

Marcinko D, Pivac N, Martinac M, Jakovljevic M, Mihaljevic-Peles A, Muck-Seler D (2007). Platelet serotonin and serum cholesterol concentrations in suicidal and non-suicidal male patients with first episode of psychosis. Psychiatry Res; 150: 105-108.

Mellman TA, Kumar AM (1994). Platelet serotonin measures in posttraumatic stress disorder. Psychiatry Res; 53: 99-101.

Mendelson SC (2000). The current status of the platelet 5-HT2A receptor in depression. J Affect Disord; 57: 13-24.

Muck-Seler D, Jakovljevic M, Pivac N (1996). Platelet 5-HT concentrations and suicidal behavior in recurrent major depression. J Affect Disord; 39: 73-80.

Muck-Seler D, Pivac N, Jakovljevic M (1999). Sex differences, season of birth and platelet 5-HT levels in schizophrenic patients. J Neural Transm; 106: 337-347.

Muck-Seler D, Pivac N, Sagud M, Jakovljevic M, Mihaljevic-Peles A (2002). The effects of paroxetine and tianeptine on peripheral biochemical markers in major depression. Prog Neuropsychopharmacol Biol Psychiat; 26: 1235-1243.

Muck-Seler D, Pivac N, Jakovljevic M, Sagud M, Mihaljevic-Peles A (2003). Platelet 5-HT concentration and comorbid depression in war veterans with or without posttraumatic stress disorder. J Affect Disord; 75: 171-179.

Muck-Seler D, Pivac N, Sagud M, Mustapic M, Jakovljevic M (2005). The effects of serotonin uptake inhibitors on platelet serotonin: From basic to clinical research. In: Trends in Serotonin uptake inhibitors research. Shirley AC, editor. NY: Nova Science Publishers, Inc., Chapter II; p. 29-53.

Mueller-Oerlinghausen B, Roggenbach J, Franke L (2004). Serotonergic platelet markers of suicidal behavior – do they really exist? J Affect Disord; 79: 13-24.

Nemeroff CB, Bremner JD, Foa EB, Mayberg HS, North CS, Stein MB (2006). Posttraumatic stress disorder: A state-of-the-science review. J Psych Res; 40: 1-21.

Nordstrom P, Samuelsson M, Asberg M, Traskman-Bendz L, Aberg-Wistedt A, Nordin C, et al. (1994). CSF 5-HIAA predicts suicide risk after attempted suicide. Suic Life Threat Behav; 24 :1–9.

Oquendo MA, Friend JM, Halberstam B, Brodsky BS, Burke AK, Grunebaum MF, et al. (2003). Association of comorbid posttraumatic stress disorder and major depression with greater risk for suicidal behavior. Am J Psychiatry; 160: 580–582.

Oxenkrug GF (1979). The content and uptake of 5-HT by blood platelets in depressive patients. J Neural Transm; 45: 285-289.

Pandey GN, Dwivedi Y, Rizavi HS, Ren XG, Pandey SC, Pesold C, et al. (2002). Higher expression of serotonin 5-HT2A receptors in the postmortem brains of teenage suicide victims. Am J Psychiatry; 159: 419-429.

Peirson AR, Heuchert JW, Thomala L, Berk M, Plein H, Cloninger CR (1999). Relationship between serotonin and the Temperament and character inventory. Psychiatry Res; 89: 29-37.

Pivac N, Jakovljevic M, Muck-Seler D, Brzovic Z (1997). Hypothalamic-pituitary-adrenal axis function and platelet serotonin concentrations in depressed patients. Psychiatry Res; 73: 123-132.

Pivac N, Muck-Seler D, Barisic I, Jakovljevic M, Puretic Z (2001). Platelet serotonin concentration in dialysis patients with somatic symptoms of depression. Life Sci; 68: 2423-2433.

Pivac N, Muck-Seler D, Sagud M, Jakovljevic M (2002). Platelet serotonergic markers in posttraumatic stress disorder. Prog Neuropsychopharmacol Biol Psychiatry; 26: 1193-1198.

Pivac N, Muck-Seler D, Sagud M, Jakovljevic M, Mustapic M, Mihaljevic-Peles A (2003). Long-term sertraline treatment and peripheral biochemical markers in female depressed patients. Prog Neuropsychopharmacol Biol Psychiatry; 27: 759-765.

Pivac N, Mück-Šeler D, Mustapić M, Nenadić-Šviglin K, Kozarić-Kovačić D (2004) Platelet serotonin concentration in alcoholic subjects. Life Sci; 76: 521-531.

Pivac N, Kozaric-Kovacic D, Mustapic M, Dezeljin M, Borovecki A, Grubisic-Ilic M, et al. (2006a). Platelet serotonin in combat related posttraumatic stress disorder with psychotic symptoms. J Affect Disord; 93: 223-227.

Pivac N, Kozaric-Kovacic D, Muck-Seler D (2006b). Biological markers in Croatian war veterans with combat related posttraumatic stress disorder. In: Novel approaches to the diagnosis and treatment of posttraumatic stress disorder. NATO Security through Science series E: Human and Societal Dynamics – Vol 6. Section 1: Epidemiology and pathophysiology of PTSD. Roy M, editor. IOS Press Amsterdam; p. 3-12.

Popova NK (2006). From genes to aggressive behavior: the role of serotonergic system. Bioassays; 28: 495-503.

Price RK, Risk NK, Haden AH, Lewis CE, Spitznagel EL (2004). Post-traumatic stress disorder, drug dependence, and suicidality among male Vietnam veterans with a history of heavy drug use. Drug Alcohol Depend; 76: 31–43.

Rao ML, Braunig P, Papassotiropoulos A. (1994). Autoaggressive behavior is closely related to serotonin availability in schizoaffective disorder. Pharmacopsychiatry; 27: 202-206.

Roggenbach J, Mueller-Oerlinghausen B, Franke L, Uebelhack R, Blank S, Ahrens B (2007). Peripheral serotonergic markers in acutely suicidal patients. 1. Comparison of serotonergic platelet measures between suicidal individuals, non-suicidal patients with major depression and healthy subjects. J Neural Transm; 114: 479–487.

Roy A, Linnoila M (1988). Suicidal behavior, impulsiveness and serotonin. Acta Psychiatr Scand; 78 :529–535.

Stahl SM (1985). Platelets as pharmacological models for the receptors and biochemistry of monoaminergic neurons. In: Platelets: Physiology and Pharmacology, Longenecker GL, editor. Academic Press, New York; p. 307-340.

Stahl SM (1998). Mechanism of action of serotonin selective reuptake inhibitors. Serotonin receptors and pathways mediate therapeutic effects and side effects. J Affect Disord; 51: 215-235.

van Praag HM (2004). The cognitive paradox in posttraumatic stress disorder: a hypothesis. Prog Neuropsychopharmacol Biol Psychiatry; 28: 923-935.

Weathers FW, Keane TM, Davidson JRT (2001). Clinician-administered PTSD scale: A review of the first ten years of research. Depress Anxiety; 13: 132-156.

Table 1. Age and clinical scores in male patients

Variables	Suicidal PTSD (N =73)	Non-suicidal PTSD (N = 47)	Suicidal non-PTSD (N =45)	Non-suicidal non-PTSD (N =30)
Age (years)	39.13 ± 7.83	40.91 ± 8.29	40.15 ± 9.56	35.33 ± 10.67
CAPS total	82.13 ± 13.60	78.85 ± 12.11	0	0
HDRS total	22.13 ± 4.45	19.73 ± 4.45	23.90 ± 3.92	12.91 ± 7.91*,**
HDRS item 3	1.84 ± 0.97	0	1.32 ± 0.65***	0
HAMA total	25.10 ± 6.62	22.51 ± 5.86•	24.78 ± 7.67	16.21 ± 8.52••
PANSS total	87.75 ± 19.96	76.42 ± 19.18#	79.48 ± 17.92	60.49 ± 21.09##

CAPS, Clinician Administered PTSD Scale; HDRS, Hamilton Depression Rating Scale; HDRS item 3, Hamilton Depression Rating Scale item for suicidal behavior; HAMA, Hamilton Rating Scale for Anxiety; PANSS, Positive and Negative Syndrome Scale; PTSD, posttraumatic stress disorder.

HDRS total scores: * $p<0.002$ vs. suicidal non-PTSD patients, ** $p<0.001$ vs. non-suicidal PTSD patients; ** $p<0.001$ vs. suicidal PTSD patients; HDRS item 3 scores: ***$p<0.003$ vs. suicidal PTSD patients; HAMA total scores: • $p<0.001$ vs. suicidal PTSD patients; •• $p<0.002$ vs. suicidal non-PTSD patients; PANSS total scores: # $p<0.036$ vs. suicidal PTSD patients; ## $p<0.001$ vs. suicidal non-PTSD patients (Tukey's test).

Legend to the Figures:

Fig. 1: Platelet 5-HT concentration (mean ± SD) in suicidal and nonsuicidal male veterans with PTSD, male suicidal and nonsuicidal comparative patients without PTSD, and in male healthy controls. * p<0.029 vs. non-suicidal PTSD patients; ** p<0.001 vs. healthy control men; # p<0.020 vs. suicidal non-PTSD patients; ## p<0.013 vs. healthy control men, Tukey's test; 95% confidence intervals (CI) for 5-HT values: healthy men (CI=1.22-1.31); suicidal veterans with PTSD (CI=0.93-1.12); non-suicidal war veterans with PTSD (CI=1.03-1.39); suicidal non-PTSD patients (CI=0.96-1.23); non-suicidal non-PTSD patients: (CI=1.14-1.48).

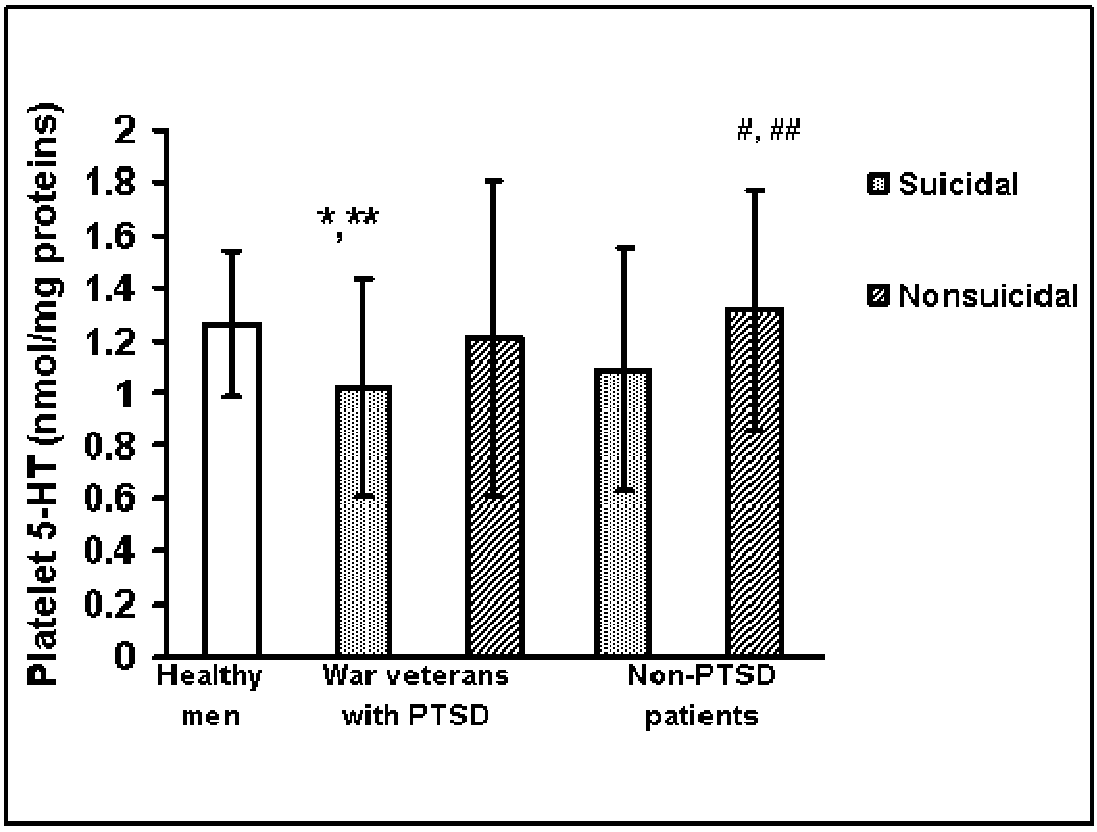

Fig. 2: Platelet 5-HT concentration (mean ± SD) in male veterans with PTSD and male patients without PTSD, subdivided into smokers and nonsmokers. 95% confidence intervals (CI) for 5-HT values: patients with PTSD – smokers (CI= 0.93-1.26); patients with PTSD – non-smokers (CI= 1.06-1.53); non-PTSD patients – smokers: (CI= 0.98-1.43); non-PTSD patients – non-smokers: (CI= 0.99-1.25).