Eur Arch Psychiatry Clin Neurosci (1991) 241:85–87

European Archives of Psychiatry and Clinical Neuroscience

Platelet Imipramine Binding in Intensive Care Unit Suicidal Patients

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Received August 20, 1990

Summary. 3H-Imipramine binding in relation to serotonergic function was studied in patients hospitalized following a suicide attempt. Comparison with a control group showed a highly significant difference in Bmax values. The results suggest, at least a posteriori, a biological alteration as the basis of suicidal behaviour. Although it is impossible to attribute a suicidal attempt to the alteration or to a predisposing pre-existent condition, this biological marker may be useful in evaluating suicidal risk and also short- and long-term prognosis.

Key words: 3H-Imipramine binding sites – Attempted suicide – Biology of suicide

Introduction

Biological research related to suicide has been closely associated with the biological aspects of depression, particularly with a physiopathological hypothesis. This is especially evident with serotonin, the cerebral metabolite of which, 5-hydroxyindoleacetic acid (5-HIAA), is reduced in the CSF (cerebrospinal fluid) of seriously depressed individuals [1]. The presence of low levels of 5-HIAA in relatives of depressed patients and in healthy control subjects has suggested that such alteration should be considered marker of vulnerability rather than an index of active pathology [2]. Furthermore, the frequently found low levels of 5-HIAA in patients with a marked proneness to suicidal behaviour [1] and the empirical evidence of a relationship between aggressiveness/hostility and suicide [3] render a physiopathological interpretation of suicide on the basis of an altered serotonergic system more acceptable [4].

The recognised similarity between platelets and serotonergic neurons and the demonstration of low- and high-affinity binding sites for imipramine on platelets has rendered possible the investigation of serotonergic function [5]. To date, the changes in parameters evidenced through the 3H-Imipramine binding (sites) has been studied in various conditions. In particular, a reduction in density of binding sites (Bmax) has been observed in patients with a diagnosis of depression when compared with healthy controls [6].

The variation in the results obtained through numerous studies induced us to reconsider the complexity of the serotonergic system (and the several factors which could influence its evaluation) and the possibility of identifying some biologically distinct subclasses within the context of diagnostic categories.

Langer and Raisman [7] suggested that the decrease in platelet Bmax could represent a biological marker for depression and that it could reflect the proneness to that pathology.

Few studies have investigated these parameters in relation to suicide. Wagner et al. [8] found an increase in Bmax in depressed patients who attempted suicide using violent methods. Roy et al. [9] found a relationship between the decrease in Bmax for imipramine, the uptake of serotonin and the probability of committing suicide.

With such premises, the possibility of applying such a study to patients who attempt suicide, appears interesting. The aim was to detect a possible proneness marker or an instrument for further assessment useful in identifying those suicidal patients who are at risk.

Subjects and Methods

Of the 15 tested patients 13 were females and 2 males. All were inpatients hospitalized in an intensive care unit following their attempt. Their mean age was 35 years (range 20–58). In all cases the blood samples were taken 2 days after the suicidal act; in 13 cases the suicidal attempt was by ingesting an overdose of benzodiazepines and in the remaining 2 cases of violent methods (gun shot and cutting of veins).

Prior to their attempt, all subjects were not receiving any drugs. The diagnosis on admission in 9 subjects were a major depressive episode, whereas 6 subjects presented with a personality disorder. In the latter group, 1 was a second parasuicide while for all the others it was a first episode.

The controls consisted of 13 subjects (11 females and 2 males), their mean age was 27.7 years, all being healthy and drug-free.

Blood samples from both experimental and control group were taken and analysed at the same time.

Table 1. Imipramine binding parameters in Suicide attempters and controls

Suicide attempters	Diagnosis	Age (years)	Bmax = fmol/mg protein	Kd = n*M*	Healthy controls	Age (years)	Bmax = fmol/mg protein	Kd = n*M*
1	M.D.E.	43	289	1.03	1	28	975	1.10
2	M.D.E.	20	323	1.40	2	26	1087	0.88
3	M.D.E.	47	499	2.08	3	28	1093	2.20
4	M.D.E.	35	636	2.20	4	28	808	1.81
5	M.D.E.	44	395	1.08	5	29	1255	2.40
6	M.D.E.	39	416	2.50	6	30	993	1.98
7	M.D.E.	32	449	0.37	7	30	1344	1.90
8	M.D.E.	24	568	2.03	8	24	1218	1.83
9	M.D.E.	58	418	1.44	9	29	1178	2.50
10	P.D.	25	301	1.69	10	24	687	1.12
11	P.D.	44	314	1.05	11	42	528	1.10
12	P.D.	26	285	0.98	12	22	504	0.97
13	P.D.	22	201	1.78	13	20	716	1.97
14	P.D.	38	313	1.54				
15	P.D.	28	387	1.54				

M.D.E. = Major Depressive Episode; P.D. = Personality Disorder

The procedure to determine the 3H-Imipramine binding on platelets was that proposed by the WHO in its 1983 protocol [10]. Blood samples of 30 ml were drawn between 07:00 and 09:00 A.M. using plastic test tubes containing 4 ml of anticoagulant with 0.38% sodium citrate (final concentration). The platelet-enriched plasma was obtained by centrifugation at 200 *g* for 20 min at room temperature. The sample was then centrifuged at 10,000 *g* for 10 min at 4°C. The supernatant was discarded and the residual pellet was suspended in 8 ml of buffer I (Tris 50 m*M*, NaCl 150 m*M*, EDTA 20 m*M*, pH 7.5), mixed and centrifuged at 10,000 *g* for 10 min at 4°C. This procedure was then repeated twice. The platelets were obtained after suspension in 8 ml of buffer II (Tris 5 m*M*, EDTA 5 m*M*, pH 7.5) for homogenization and centrifugation at 20,000 *g* for 10 min at 4°C. The resulting pellet was suspended in 8 ml of buffer III (Tris 70 m*M*, pH 7.5), mixed and centrifuged as above. The resulting platelet pellet could then be stored at −80°C for use after suspension in 3.5 ml of buffer IV (Tris 50 m*M*, NaCl 120 m*M*, KCL 5 m*M*, pH 7.4) and homogenization.

Aliquots of platelet membrane (100 µl) were then incubated for 2 h at 0°C with 50 µl of 3H-imipramine in progressively increased concentrations (0.30–40 n*M*), 150 µl of desipramine 200 µ*M* or buffer IV to a final volume of 300 µl. Binding was determined by three successive rapid filtrations with 5 ml of buffer IV at 0°C. The protein concentration of the samples was evaluated by Peterson's method [11]. Bmax (site density) and Kd (dissociation constant) values were calculated by Scatchard's linear regression method.

Results

The specific binding of imipramine on human platelets reached saturation with concentrations ranging from 10 to 15 n*M*; the use of a wider range (0.3 to 40 n*M*) gave complete saturation curves and avoided possible overestimation of Bmax and Kd during data extrapolation [13]. Table 1 summarizes the findings.

The binding was found to be roughly 70% of the total. In suicide attempters the mean value of Bmax was 386.3, SD 116.5 and was less than the value in the control group (952.8, SD 279.1) indicating a very significant difference i.e., $P < 0.001$ ($t = 7.188$).

Table 2. Comparison between suicide attempters and controls

	Bmax		Kd	
Suicide attempters	386.3	(SD 116.5)	1.5	(SD 0.56)
Controls	952.8	(SD 279.1)	1.7	(SD 0.57)

$t = 7.188$; $P < 0.001$

As regards Kd, the mean value recorded in both groups did not show a significant difference (1.5 SD 0.56 and 1.7 SD 0.57 respectively), ($P = 0.46$, $t = 0.749$), (Table 2).

In the given range, no correlation was found between the age of the subjects and Bmax values. Within the group of suicide subjects in significant differences in Bmax and Kd were found between patients having a diagnosis of depression (N = 9) and patients with a diagnosis of personality disorder (N = 6). (Mann-Withney U = 49 and U = 31 respectively).

Discussion and Conclusions

The binding of 3H-imipramine on human platelets has been widely applied to the study of serotonergic function, both in psychiatric and non-psychiatric conditions. The somewhat varying results reveal the complexity in evaluating the serotonergic system and the possible effects due to the assessment methods [12]. For example, the possibility of an overestimate of real value due to a lack of saturation in the curves, especially that of Bmax, has been observed [13]. Therefore, we increased the concentration range to reach a complete saturation of binding sites aiming to obtain a more effective measurement of the parameters.

Agreeing with the literature on Bmax alteration in depressed subjects [14] and on cortical receptors modification of serotonin in suicide victims [15], our data revealed a clear difference between suicide attempters and healthy controls.

This highly significant difference seems to indicate, at least a posteriori, the existence of a biological alteration as the basis of such suicide behaviour. Clearly it is not yet possible to establish if this alteration could be due to an acute condition following deliberate self-harm or to a pre-existing general proneness.

As reported in earlier work, the reduction of Bmax could be due to the presence of a depression which gave rise to the suicidal act or to the presence of other psychological problems. On the other hand, it is also possible that this serotonergic alteration could be specifically related to a particularly suicide-prone diagnostic subgroup.

More specifically, the presence of subjects in the sample with different diagnoses (major depression and personality disorder) but with similar changes in the serotonergic system could suggest an altered control of aggressiveness in relation to the self-harming tendency but relatively independent of the actual pathological condition. This is confirmed by the lack of a significant difference between the two sub-groups of subjects diagnosed with major depression and personality disorder, even though the small number of subjects involved in the sample (9 as compared with 6) does not allow us to draw any definite conclusion. Furthermore, the high percentage of suicidal repeaters, even after a brief period, could in itself be important in the difficult assessment of suicidal risk, besides the evaluation of short- and long-term prognosis. Bearing in mind that suicidal subjects who use benzodiazepines are not taken so seriously by intensive care units and emergency room physicians, and that 10% of them will later die because of suicide [16], the importance of detecting a biological marker, such as the reduced Bmax, could represent an important tool in the prevention of suicidal behaviour.

Finally, one should consider the possibility that in the suicide attempters group, Bmax was found to be affected by benzodiazepine overdose. However, studies using the same methods in patients with chronic treatment with benzodiazepines and also those with short-term treatment using high doses (e.g. 20 mg of diazepam), have excluded changes in Bmax (L. Traskman-Bendz, personal communication). Furthermore, Table 1 shows that the two subjects with violent suicide attempts (14 and 15) had Bmax values which were indistinguishable from those of subjects with a benzodiazepine overdose.

Obviously, further studies should be directed both to improve the technique and the understanding of the correlations between biological changes and clinical/personality aspects. Naturally, only by extending the research and follow-up of these subjects could our preliminary findings be confirmed.

Also, the possibility of applying this biological marker as a prognostic factor or as a parameter not only of suicide risk, but also of primarily assessing the efficacy of objective therapeutic and preventive measures will be of considerable interest.

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