The Effect of Psychoactive Drugs on in vitro Platelet Function

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Whole blood platelet aggregation · Impedance aggregometry · Neuroactive psychoactive drugs · Neuronal receptors

Summary
Background: Neuro-hormonal and hemostatic mechanisms are important in a wide range of psychological and cardiovascular diseases. The use of psychoactive drugs in mental illnesses is often involved with hematologic side effects including impaired platelet function. Subsequently, the risk for the development of cardiovascular diseases may be higher in these patients. Interestingly, platelets that play a key role in cardiovascular complications contain quite a number of neuronal receptors which are involved in psychotic disorders. It has been widely discussed whether psychoactive drugs used in the therapy of psychotic disorders have a direct effect on platelet function and whether the effects are transmitted through the corresponding receptors on the platelet surface. Material and Methods: In this study, we tested several psychoactive drugs regarding their impact on whole blood platelet aggregation. Results: Antidopaminergics preferentially inhibited ADP-induced aggregation whereas anticholinergics mainly inhibited U46619-induced aggregation. Because platelets respond selectively to different psychoactive drugs we assume that corresponding receptors have a functional aspect on platelets and that receptor blockade affects platelet aggregation through different mechanisms. Conclusion: The knowledge about the effects of psychoactive drugs on platelet function may help to characterize neuronal receptors on platelets and may contribute to a better understanding of altered platelet function during therapy with psychoactive drugs.

Schlüsselwörter
Vollblutthrombozytenaggregation · Impedanzaggregometrie · Neuroaktive psychoaktive Medikamente · Neuronale Rezeptoren

Zusammenfassung
Hintergrund: Neurohormonale und hämostatische Mechanismen sind für die Entstehung zahlreicher psychologischer und kardiovaskulärer Erkrankungen von großer Bedeutung. Zahlreiche Studien der letzten Jahre belegen, dass die Behandlung mentaler Erkrankungen mit psychoaktiven Medikamenten mit Thrombozytenfunktionsstörungen einhergehen kann. Folglich könnte bei diesen Patienten das Risiko für die Entstehung von kardiovaskulären Erkrankungen steigen. Interessanterweise verfügen Thrombozyten, die bei der Entstehung kardiovaskulärer Komplikationen von zentraler Bedeutung sind, über eine Vielzahl von Rezeptoren, die funktionell an psychotischen Störungen beteiligt sind. Seit längerem wird diskutiert, ob psychoaktive Substanzen, die in der Therapie psychotischer Störungen eingesetzt werden, einen direkten Einfluss auf die Thrombozytenfunktion haben und ob die Effekte eventuell durch die entsprechenden Rezeptoren vermittelt werden. Material und Methoden: In der vorliegenden Studie haben wir den Einfluss verschiedener psychoaktiver Substanzen auf die Thrombozytenaggregation im Vollblut getestet. Ergebnisse: Antidopaminergische Substanzen inhibierten signifikant die ADP-induzierte Aggregation, während anticholinergische Substanzen substitutiv die durch Thromboxan A2 (U46619) induzierte Aggregation hemmten. Da die Thrombozyten also selektiv unterschiedlich auf die Substanzen reagieren, ist zu vermuten, dass die entsprechenden Rezeptoren bei den Thrombozyten vorhanden sind und deren Blockierung sich durch unterschiedliche Mechanismen auf die Thrombozytenaktivierung auswirkt. Schlussfolgerung: Die Erkenntnisse über die Wirkung psychoaktiver Substanzen auf die Thrombozytenfunktion können sowohl zur funktionellen Charakterisierung neuronaler Rezeptoren bei Thrombozyten als auch zum Verständnis veränderter Thrombozytenfunktion während der Therapie mit psychoaktiven Substanzen beitragen.
Introduction

Epidemiologic, clinical, and laboratory studies have repeatedly demonstrated a relationship between the cardiovascular system and stress, psychological symptoms, and psychiatric disorders [1, 2]. The use of psychoactive drugs in mental illnesses is often involved with hematologic side effects including impaired platelet function [3]. Many neuronal molecules and receptors, which are involved in the neuronal mechanism of mental diseases, have also been shown to be involved in platelet function. These include G-protein-coupled receptors (GPCRs), e.g. serotonin, catecholamine, and tachykinin receptors, and as recently demonstrated those which form ion channels, e.g. glutamate receptors [4–6]. Most of the knowledge about the non-neuronal system on platelets comes from their use as a peripheral model for the investigation of the efficacy of psychoactive drugs in mental illnesses [2, 7]. In this respect, it has been a long discussion whether the effects of psychoactive drugs on platelet function are rather caused by unspecific membrane disturbance than by specific binding to corresponding receptors on platelets [2, 7].

Platelets express a complete and functional dopaminergic system including the dopamine transporter (DAT) and dopamine receptors [8, 9]. Recently, we showed that dopamine can potentiate ADP-induced platelet microaggregate adhesion through D2-like dopamine receptors [10]. A number of D2-like dopamine receptor antagonists, e.g. clozapine and raclopride, are currently in use for the treatment of schizophrenic patients. An association between D2-like dopamine receptor antagonists and modulation of platelet function was frequently reported, and most of these in vivo reports concern clozapine [11, 12]. A relationship between D2-like receptors and depression has recently been reviewed [13].

The effect of cholinergic drugs on platelet function has not been reported, up until now. Most of the cholinergic molecules target nicotinic ion channels including the non-competitive antagonist mecamylamine [14]. Mecamylamine has been clinically used for the treatment of hypertension, depression, autism, and alcohol abuse. Furthermore, in developing smoking cessation strategies, mecamylamine has been shown to be useful when co-administered with transdermal nicotine [15–18]. In line with this, the opioid receptor inhibitor naltrexone, a well-known competitive narcotic antagonist used in the maintenance treatment of acute opioid intoxications, was also reported to be a beneficial co-adjuvant in smoking cessation programs [19]. Interestingly, naltrexone was shown to suppress platelet aggregation in former heroin addicts [20].

These reports and the increasing knowledge about functional neuronal receptors on platelets encouraged us to investigate the effect of psychoactive molecules on ex vivo platelet function. We investigated psychoactive drugs of the dopaminergic system known to modulate platelet function and cholinergic drugs including the anti-opioid (naltrexone). These have not been investigated up until now for this purpose. Using the MultiplateTM analyzer, we measured platelet aggregation in whole blood of healthy, non-smoking volunteers. Platelet activation via different receptors and signaling pathways was achieved by application of classical platelet agonist ADP (P2Y1/P2Y12 receptors and GpIb/IIa protein signaling) and U46619 (thromboxane A2 receptor and GpIIb/IIIa protein signaling) [21, 22]. In order to prove the suitability of the testing method used, we also applied the calcium channel blocker verapamil since its antiplatelet effect is well documented in literature [23].

Material and Methods

Drugs and Chemicals

ADP (ADPtest, 2.5 μmol/l) was purchased from Dynabte (Munich, Germany). U46619, S(-)-raclopride (+)-tartrate salt, clozapine, mecamylamine hydrochloride, and naltrexone hydrochloride were from Tocris Bioscience (Ellisville, MI, USA). Verapamil hydrochloride, methyllycaconitine (MLA) citrate, and atropine were from Sigma-Aldrich (Taufkirchen, Germany).

Multiple Electrode Aggregometry

According to the manufacturer’s instructions, recombinant hirudin (25 μg/ml) was used as anticoagulant (hirudin blood collection tubes; Dynabyte, Munich, Germany). Whole blood was donated by 6 healthy, non-smoking individuals, who had not taken any medication known to interfere with platelet function for the last 10 days. The study volunteers gave their informed written consent for using their blood samples for platelet in vitro studies. Platelet aggregation was determined using multiple electrode aggregometry (MEA) on the multichannel Multiplate™ analyzer (Dynabyte). 300 μl of whole blood were added to the MEA test cell and warmed for 3 min to a temperature of 37 °C. The activator solutions were added and aggregation was measured over a time period of 6 min. For the investigation of psychoactive drugs a blood sample was pre-incubated with the desired substance for 30 min before applying the sample to aggregometry. The impedance change due to the attachment of platelet to cell and warmed for 3 min to a temperature of 37 °C. The activator solutions were added and aggregation was measured over a time period of 6 min. For the investigation of psychoactive drugs a blood sample was pre-incubated with the desired substance for 30 min before applying the sample to aggregometry. The impedance change due to the attachment of platelets to the sensor electrodes was quantified and given as arbitrary units (AU). In order to avoid overstimulation of platelets by exceeding agonist concentrations, the minimal agonist concentration for significant platelet aggregation was determined in a single pilot experiments for ADP as 2.5 μmol/l and for U46619 as 0.75 μmol/l.

Statistical Analysis

Each substance was tested in at least 6 healthy individuals. Statistical analysis was performed with a t test analysis for paired samples (aggregation without psychoactive drug vs. aggregation with drug) by using SPSS 12.0 statistical software (SPSS, Chicago, IL, USA). P-values <0.05 were considered as significant.

Results

In this study we investigated psychoactive drugs, which either act on GPCRs or calcium channels as described for antidopaminergics and anticholinergics, respectively. In order to evaluate the capability of the Multiplate™ analyzer to detect antiplatelet effects in vitro, we examined the calcium channel blocker verapamil and the GPCR antagonist clozapine.
Effect of Antidopaminergics on Whole Blood Platelet Aggregation

In previous studies, our group identified dopamine D2-like receptors to be involved in ADP-mediated platelet activation [10]. Therefore, we expected dopamine D2-like antagonists to affect ADP-induced platelet aggregation. We tested the D2-like receptor antagonists, clozapine and raclopride, on ADP- and U46619-induced platelet aggregation. As expected, ADP-stimulated aggregation was significantly inhibited by clozapine at 10 μmol/l (to 63 ± 13 AU; p < 0.001; fig. 2). Raclopride at 1 μmol/l tended to inhibit aggregation.

Fig. 1. Effect of psychoactive drugs on U46619-induced platelet aggregation: Whole blood of 6 healthy, non-smoking individuals was pre-incubated for 30 min with the calcium channel blocker verapamil (VER; 100 nmol/l); the antidopaminergics clozapine (CLO; 10 μmol/l) and raclopride (RAC; 1 μmol/l); the anticholinergics atropine (ATR; 1 μmol/l), mecamylamine (MECA; 10 μmol/l) and methyllycaconitine (MLA; 100 nmol/l), and the anti-opioid naltrexone (NE; 25 μmol/l) prior to stimulation with the classical platelet agonist U46619 (0.75 μmol/l). All drugs were used in therapeutically relevant concentrations. VER, CLO, MLA, and NE significantly inhibited U46619-induced platelet aggregation. The other drugs tended also to inhibit platelet aggregation which, however, did not reach statistical significance.

Fig. 2. Effect of psychoactive drugs on ADP-induced platelet aggregation: Whole blood of 6 healthy, non-smoking individuals was pre-incubated for 30 min with the calcium channel blocker verapamil (VER; 100 μmol/l); the antidopaminergics clozapine (CLO; 10 μmol/l) and raclopride (RAC; 1 μmol/l); the anticholinergics atropine (ATR; 1 μmol/l), mecamylamine (MECA; 10 μmol/l) and methyllycaconitine (MLA; 100 nmol/l), and the anti-opioid naltrexone (NE; 25 μmol/l) prior to stimulation with the classical platelet agonist ADP (2.5 μmol/l). All drugs were used in therapeutically relevant concentrations. VER, CLO, and ATR significantly inhibited ADP-induced platelet aggregation. The other drugs tended also to inhibit platelet aggregation which, however, did not reach statistical significance.

Effect of Verapamil on Whole Blood Platelet Aggregation

The antplatelet action of verapamil is well documented in the literature [23]. Pre-treatment of whole blood with a rather low concentration of verapamil (100 nmol/l) resulted in a significant inhibition of U46619-induced platelet aggregation (145 ± 18 AU to 76 ± 11 AU; p = 0.01; fig. 1) but not ADP-induced aggregation (data not shown). However, at a 1,000-fold higher concentration of verapamil (100 μmol/l), the ADP-induced aggregation was also inhibited (151 ± 18 AU to 98 ± 25 AU; p = 0.001; fig. 2).

Psychoactive Drugs and Platelet Function
Discussion

The results of this study clearly demonstrate that psychoactive drugs of the dopaminergic and the cholinergic type have an influence on platelet function (summarized in table 1). We could also show that whole blood impedance aggregometry (performed by using the Multiplate™ analyzer) represents a suitable assay to detect drug-induced platelet inhibition. This was demonstrated using the calcium channel blocker verapamil, which has been extensively studied regarding its antiaggregatory effects on platelets [23]. Furthermore, it was reported that low concentrations of verapamil cause inhibition of transmembrane calcium influx, whereas inhibition of ADP-induced calcium mobilization from intracellular stores requires higher concentrations of the drug [26]. In our study, low concentrations of verapamil (100 nmol/l) inhibited U46619-induced aggregation, which could be assigned to transmembrane calcium influx. At a 1,000-fold higher concentration of verapamil, ADP-induced aggregation was also inhibited, which supports previous data [26]. We assume that calcium channel antagonists mainly target U46619-induced platelet aggregation because U46619 at lower concentrations, as used in our study, mainly mediates calcium-related mechanism in platelets.

Although the non-competitive cholinergic antagonist mecamylamine had no effect, the competitive cholinergic antagonist MLA significantly inhibited U46619-induced aggregation, but not ADP-induced aggregation. Since MLA is a structural prototype for the identification of novel cholinergic drugs, this inhibitory action of MLA may prove to be important [24]. In addition, the opioid receptor antagonist naltrexone, which was reported to bind to cholinergic ion channels in neurons, also inhibited U46619-induced but not which did not reach significance (to 126 ± 25 AU; p = 0.1). Similarly, U46619-induced aggregation was significantly inhibited by clozapine (to 94 ± 26; p = 0.033), whereas raclopride had no effect (fig. 1).

### Table 1. Psychoactive drugs and their effects on in vitro platelet aggregation: summary of the study results

<table>
<thead>
<tr>
<th>Substance group</th>
<th>Substance</th>
<th>Receptor specificity</th>
<th>Clinical use</th>
<th>Inhibition of U46619-induced aggregation*</th>
<th>Inhibition of ADP-induced aggregation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Type channel blocker</td>
<td>verapamil</td>
<td>L-Type Ca2+ channels</td>
<td>hypotension, cardiac arrhythmia</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Antidopaminergics</td>
<td>clozapine</td>
<td>D2-like dopamine receptors</td>
<td>schizophrenia</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Antidopaminergics</td>
<td>raclopride</td>
<td>D2-like dopamine receptors</td>
<td>schizophrenia</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Anticholinergics</td>
<td>atropine</td>
<td>muscarinic AChRs</td>
<td>anaesthesia</td>
<td>−</td>
<td>++</td>
</tr>
<tr>
<td>Anticholinergics</td>
<td>mecamylamine</td>
<td>nicotinic AChRs</td>
<td>alcohol dependence, smoking cessation</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Anticholinergics</td>
<td>methyllycaconitine</td>
<td>nicotinic AChRs</td>
<td>in clinical trials</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Anti-opioids</td>
<td>naltrexone</td>
<td>opioid receptors</td>
<td>alcohol dependence, smoking cessation</td>
<td>++</td>
<td>−</td>
</tr>
</tbody>
</table>

*A inhibitory effects of psychoactive drugs on U46619- or ADP-induced platelet aggregation were significant (+: p < 0.05; ++: p < 0.01) or not detectable (−: p > 0.05). AChR = Acetylcholine receptor.
ADP-induced aggregation. This suggests an association between ion channels and thromboxane receptor signaling in platelets.

Based on our previous findings on dopamine co-agonism [10], we expected the D2-like receptor antagonist clozapine to inhibit ADP-induced aggregation. Indeed, the most pronounced inhibition by clozapine was seen for ADP-induced aggregation. The D2-like receptor antagonist raclopride showed an inhibitory tendency. Interestingly, the cholinergic antagonist atropine, which targets G-protein-coupled muscarinic acetylcholine receptors inhibited ADP-induced but not U46619-induced platelet aggregation. These results imply that different psychoactive drugs affect different platelet activation pathways. The question arises whether these effects are mediated via direct inhibition of the respective drug receptors on platelets. At least for the dopaminergic system this question can be answered: platelets do possess a complete functional dopaminergic system [8–10], and the inhibitory effect seen by antidopaminergics is very likely a receptor-dependent mechanism. Based on microarray data, we have identified cholinergic receptor gene transcripts in platelets of the nicotinic and the muscarinic type [27–29]. In addition, it has been suggested that platelets store acetylcholine in their granules and acetylcholine esterase is present on platelets [30–31]. Together with our data on platelet inhibition by anticholinergics this indicates a cholinergic system on platelets.

The psychoactive drugs tested in this study are frequently used in the therapy of psychotic disorders. Further knowledge about the effects of the drugs on platelet function may help to understand pathomechanisms that cause a significantly higher risk for the development of cardiovascular disease in patients with psychotic disorders. In addition, our results may be of importance for cardiovascular patients that are under cardiovascular surgery. These patients may be under dual platelet therapy with aspirin and clopidrogel. We suggest close monitoring of platelet function in cardiovascular patients under dual platelet therapy, especially when they are also under treatment with psychotic drugs. The platelet inhibitory properties of anticholinergics may also contribute to the development of new antiplatelet drugs and the identification and characterization of cholinergic receptors on platelets.

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Disclosure

The authors declared no conflict of interest.

References


