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RESEARCH NOTE

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Effect of D-serine on the serotonin receptors of human platelets

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Abstract Recent literature and our previous observations indicated the presence of both NMDA and serotonin type 3 receptors in human platelets with very similar ionic currents to that of cultured mammalian neuronal receptors. Baseline electrophysiological data shows similar profile for platelets from both normal and schizophrenic subjects, whereas serotonin receptor studies exhibited the presence of 5-hydroxytryptamine type-3 (5-HT3) currents in both normal and schizophrenic platelets significantly different from each other. The two major differences observed were: first, 5-HT3 receptors present in the platelets of schizophrenic patients were four times more sensitive to serotonin than those present in the platelets of normal subjects and, second, that D-serine in micro molar concentrations dampens this effect in platelets from schizophrenic patients but increases the sensitivity of serotonin for platelet 5-HT3 receptors of normal subjects. Patch clamp technique was used to measure the whole cell currents passing through serotonin receptors in these two types of human platelets. The currents were found to be 5-HT3 receptor currents as they were abolished by 10 μM D-tubocurarine. Similarly, micromolar concentrations of D-serine increased the sensitivity of 5HT3 receptor currents in the normal human platelets but decreased it in the platelets of the schizophrenic patients. This effect was reversed when D-amino acid oxidase (0.3 μM) was co applied with 100 μM of D-serine, raising the possibility that D-serine by itself may act as a modulator for platelet 5-HT3 receptor channel currents. These observations raised exciting new questions about the role of platelet serotonin receptors and their regulation by D-serine.

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Introduction

Platelets are reliable biological markers for neuronal activities (Borges et al. 2004) and related disorders (Aspey et al. 1997). Recent literature and our initial studies indicated that platelets have similar serotonin and glutamate receptors like those in neurons and they are capable of interacting (Harsing et al. 2004).

Degenerative neurological disorders are associated with glutamate anomalies (Kostic et al. 2003). Similarly, serotonin receptors of human platelets also proved to be a good model for neuronal 5-HT receptor pathologies (Christopher et al. 1995).

A new enzyme has recently been discovered in the brain that converts L-serine into 'D-serine', which has an important function in modulating the brain electrical circuitry by acting as co-agonist for glutamate-gated NMDA receptors (Miranda et al. 2002). D-serine is an endogenous ligand and its depletion in brain slices and cultured neuronal cells strongly diminishes NMDA receptor responses measured biochemically and electrophysiologically (Mothet et al. 2000a, b).

A series of studies on more than 350 psychotic and non-psychotic subjects revealed that serine concentrations were higher in plasma from psychotic patients (including schizophrenia) compared to control subjects (Waziri et al. 1983) and anomalies of platelet receptors (Michal 1969) would be a good indicator for modeling schizophrenia and other related mental disorders. However, it is not known whether D-serine is capable of directly interacting with serotonin receptors. Therefore, in this paper, the type of serotonin receptors present in the platelet's membrane (obtained from normal and schizophrenic subjects) has been identified and the response of these receptors to D-serine was examined.

Methods

Isolation of platelets

Whole blood (20 ml) was collected from fasted (12 h) normal subjects and drug naïve schizophrenic patients of matching age weight and gender. The diagnosis of schizophrenia was done according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) by privately practising psychiatrists. This study was approved by Human Research Ethic Committee, University of Newcastle (approval no: HE 99/347), and complied with the NHMRC national statement (1999) on ethical conduct in research involving humans. A consent form was filled and signed by each subject (both normal and schizophrenic) prior to drawing the blood.

Human platelets were isolated from fresh human blood into acid/citrate/dextrose (ACD) anticoagulant (70 mM citric acid, 85 mM sodium citrate, 110 mM D-glucose) (4 ml ACD/20 ml blood). Blood was centrifuged for 20 min at 250*g* at a temperature below 22°C. Platelets in the supernatant (Platelet-rich Plasma, PRP) were obtained by centrifugation (700*g*, 12 min). The pellet was gently resuspended in 2 ml of Tyrodes buffer and filled up to 10 ml of washing buffer (36 mM sodium citrate, 5 mM KCl, 90 mM NaCl, 10 mM EDTA, 5 mM D-glucose, 0.1 mM aspirin, pH 7.4). The platelets were then immediately transferred into bath chamber for patch clamp studies.

Electrophysiological studies of platelets using patch clamp techniques

The platelets were transferred into a bathing chamber at the stage of an Olympus IX70 inverted microscope for studying their passive electrical properties and serotonin receptor currents. Electrophysiological manipulation and recordings were undertaken with a HEKA EPC9 amplifier and HEKA Pulse software package. Thin walled borosilicate glass capillaries were used to produce patch pipettes with a 3 MΩ resistance. Pipettes were half-filled using both the front- and back-filling techniques. Solution-filled glass pipettes were attached to an Ag/AgCl recording electrode and manipulated using a PCS-5000 series patch clamp micromanipulator (Burleigh Instruments). Platelet patching was performed according to the patch clamp protocol (Hamil et al. 1981).

Two sets of bath and pipette solutions were used for this experimental series. The first set, the normal solutions, imitated the internal and external in vivo conditions, and, importantly, replicated the normal internal monovalent cation concentrations found in the platelet's native environment. The normal pipette solution contained: 120 mM KCl, 3.7 mM NaCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 20 mM TEACl, 10 mM HEPES, 11 mM EGTA, (pH 7.4), and externally to the normal bath solution contained: 137 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 2 mM $MgCl_2$, 5 mM HEPES, 10 mM D-glucose (pH 7.4).

The second set of solutions, the experimental solutions, were designed to produce a resting membrane potential of 0 mV and thus presented equal (symmetrical) cation concentrations on either side of the membrane. The experimental bath solution contained: 140 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM D-glucose, pH 7.4, and the experimental pipette solution contained: 140 mM NaF, 1 mM $MgCl_2$, 10 mM HEPES, 10 mM EGTA, pH 7.4.

A stimulus perfusion system was employed to introduce chemical stimuli onto a patched platelet with stimulation time being electronically controlled via solenoid valve regulation. The agonist solutions used in this experimental series were a set of (1–1000 μM) 5-HT hydrochloride dilutions. Patched platelets were challenged with 80,000 ms exposure to agonist at 5 min intervals and the results recorded using the HEKA PULSE software. The antagonist solution used was 5 μM D-tubocurarine chloride. Platelets were again challenged with 80,000 ms exposure, both with and without agonist solution. Whole cell recordings of serotonin currents for both types of platelets were also obtained in the presence of 100 μM D-serine and 0.3 μM D-amino acid oxidase (DAAO). It was extremely important that all work involving the agonist solutions should be carried out in the dark because light catalysed serotonin oxidization rendering it unfit for experimental use.

Results

Cells were examined via the patch-clamp technique in normal solutions where K^+ was the primary cationic component of the pipette solution. The normal solutions imitated the internal and external conditions found in vivo, and most importantly simulated the normal internal cation concentrations found in a platelet's native environment. Throughout the course of the experimental series all patch-clamp recordings were taken at a constant 24°C (room temperature activates human blood platelets, major changes occur at 20°C; they irreversibly lose discoid shape below 15°C).

In this normal bath and pipette solution, passive electrical properties, such as resting membrane potential (± − 50 mV), input resistance (± 60 GΩ) and cell capacitance (± 130 fF) were similar for platelets obtained from both normal and schizophrenic subjects (Table 1). The predominant ionic currents in both types of platelets were similar to those of voltage-gated K^+ delayed rectifier of neurons.

In the presence of experimental solutions, the calculated reversal potential (the voltage when there is zero current response, denoted E_{rev}) was 0.14 mV, and the calculated ± 30 mV slope conductance (the average conductance at + 30 mV divided by the average conductance at −30 mV) was 1.02 (Fig. 1, left panel).

In the symmetrical solutions when the platelet resting membrane potential was close to zero, serotonin in

Table 1 Passive electrical properties of platelets

Platelets (schizophrenic subjects)	Platelets (normal subjects)	Properties
-54 ± 3.14	-50 ± 2.06	Resting membrane potential (mV)
57 ± 2.13	59 ± 1.13	Input resistance (GΏ)
133 ± 1.03	128 ± 2.23	Cell capacitance (fF)
Voltage gated K+ channels similar to delayed rectifier of neurons	Voltage gated K+ channels similar to delayed rectifier of neurons	Predominant ion channel

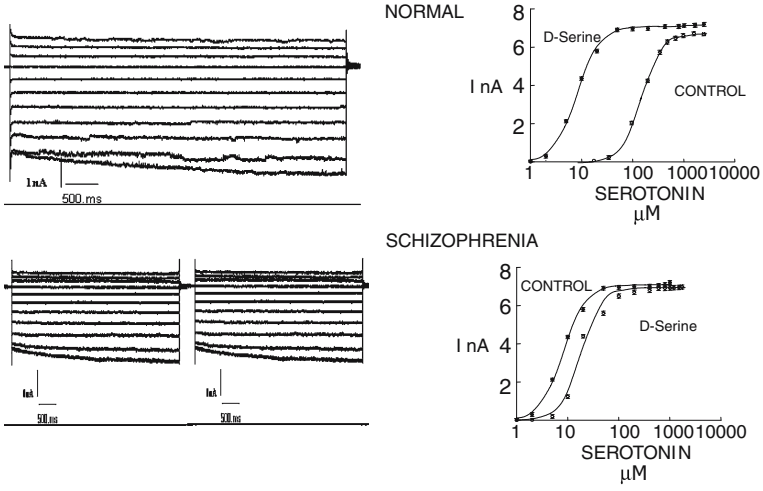

Fig. 1 Effect of D-serine on platelets 5-HT3 receptors showing I/V and D/R for both normal and schizophrenic subjects

micromolar concentrations produces fast currents of nano ampere amplitudes which were abolished in the presence of micromolar concentration of D-tubocurarine, suggesting the presence of 5-HT3 receptor channels in the platelet membranes from both normal and schizophrenic subjects.

Similarly, dose–response curves of serotonin (see Fig. 1, right panel) exhibits a significant fourfold increase ($K_d = 2.65 \pm 0.22$ μM) in the affinity of serotonin to $5HT_3$ receptors in the platelets from schizophrenic patients as compared to normal subjects ($K_d = 11.69 \pm 0.45$ μM). In the presence of micromolar concentration of D-serine, the dose–response curve for serotonin gated currents in the platelets of schizophrenic subjects was shifted to right with a K_d of 8.73 ± 0.43 μM, and for normal subjects shifted to the left giving a K_d value of 1.95 ± 0.31 μM; mean ± standard error, $n = 9$. However, when these platelet 5-HT3 receptors were exposed to co-application of 0.3 μM of DAAO and 100 μM of D-serine in the presence of 5 μM of serotonin, there was a fast current of around 1.5 nA of current (Fig. 2c), which was very similar to that of serotonin currents in the platelets of schizophrenic subjects (current not shown), demonstrating that the dampening of serotonin sensitivity (Fig. 2a) in the presence of D-serine was abolished by the presence of DAAO. The current amplitudes in Fig. 2 showing that the 500 pA current produced by 5 μM of serotonin plus 100 μM of D-serine was increased three times in the presence of 0.3 μM of DAAO for the platelet receptors of schizophrenic patients.

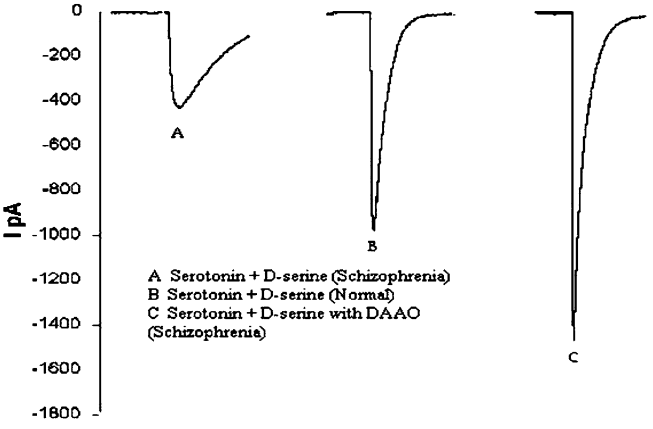

Fig. 2 5-HT3 receptor currents in the presence of D-serine and D-amino acid oxidase

Discussion

The platelet is one of the most researched biological markers in psychiatry. Characteristics of MAO activity, 5-HT uptake, 5-HT3 receptor currents, imipramine and α_2-adrenergic receptor binding, are similar in platelet and CNS (Wirz-Justice 1988). In these experiments ionic currents passing through the serotonin receptors was measured using patch clamp technique. Platelets are of 2–4 μm in diameter with a volume of 4–7.6 μm resulting

in a surface/volume ratio > 1, which is ideal for quick exchange of the internal solution with the pipette solution. They are verified to be very good models for studying receptor ionic current activities (even though patch clamping platelets was not an easy task).

In the presence of normal bath and pipette solution, platelets like those of neurons exhibit K as the main carrier for their ionic currents. The current voltage response of serotonin type 3 receptors in human platelets were slightly outward rectifying for both normal and schizophrenic subjects. The averaged maximum conductance of control platelet (0.28 nS at + 30 mV) indicates that the serotonin receptors shows their highest voltage-determined conductance at positive potentials (> 0 mV), thus displaying slight outward current rectification (positive ions move from the cellular cytoplasm into the surrounding solution). This finding was confirmed by ± 30 mV slope conductance (1.08 nS) where a slightly higher conductance was seen at + 30 mV (0.28 nS) than at −30 mV (0.26 nS).

A fast-activating increase in channel conductance in response to the addition of serotonin was observed, where an increase in 5-HT concentration resulted in a higher conductance level, so that conductance response for the platelet receptors was 1 mM 500 μM > 10 μM > 5 μM > 1 μM for control platelets giving a Vmax of 886 ± 21.5 μM with a Hill coeficient of greater than 2 for platelet receptors from normal subjects and Vmax of 435 ± 13.5 μM with a Hill coefficient greater than 2 for platelet receptors of schizophrenic subjects, indicating that in either case the serotonin receptor has atleast two binding sites to be occupied to reach the open state. On the other hand, the lower Kd values of platelet serotonin receptors of schizophrenic subjects indicated the higher residency time of serotonin for these receptors, which numbered according to their Vmax values less than half of those from normal subjects.

The passive electrical properties of both types of platelets were similar. The pathology was found to be in serotonin type 3 receptors since the binding affinities of platelet serotonin receptors from schizophrenic patients seemed to be four times greater than those of the normal receptor. As the serotonin sensitivity was brought back close to normal subject's values in the presence of D-serine, it was postulated that D-serine by itself binds to the NH3 terminal or hydrogen binds to the serotonin receptor complex and dampens the hypersensitive effect. The action was reversed in the presence of D-serine-specific enzyme DAAO, indicating that D-serine directly altered the serotonin effect. Platelets from both schizophrenic and normal subjects exhibit similar passive electrical properties but there was a huge difference of serotonin sensitivity between them indicating that anomalies are present in the 5-HT3 receptor itself. Although literature (Anat Biegon et al. 1990) indicated the presence of 5-HT2 receptors in platelets and their interaction with 5-HT3 receptors (Julius 1991) along with serotonin interacting with other bio amines in schizophrenia (Kahn and Davidson 1993), it is for the first time that the presence of 5-HT3 receptor currents has been shown in the platelets from schizophrenic and normal platelets. Similarly, glutamate and serotonin interaction is well known (Aghajanian and Marek 1999) as also the D-serine glutamate co activity (Mothet et al. 2000a, b), but the D-serine modulation of the platelet's serotonin receptors has been observed for the first time. On the basis of the aforementioned observations, addition of D-serine in the schizophrenic regime is proposed to be beneficial.

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