

Paroxetine suppresses recombinant human P2X7 responses

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Abstract

P2X7 receptor (P2X7) activity may link inflammation to depressive disorders. Genetic variants of human P2X7 have been linked with major depression and bipolar disorders and the P2X7 knockout mouse has been shown to exhibit anti-depressive like behaviour. P2X7 is an ATP-gated ion channel and is a major regulator of the pro-inflammatory cytokine interleukin 1 β (IL-1 β) secretion from monocytes and microglia. We hypothesised that antidepressants may elicit their mood enhancing effects in part, via modulating P2X7 activity and reducing inflammatory responses. In this study we determined whether common psychoactive drugs could affect recombinant and native human P2X7 responses *in vitro*. Common antidepressants demonstrated opposing effects on human P2X7-mediated responses; paroxetine inhibited while fluoxetine and clomipramine mildly potentiated ATP-induced dye uptake in HEK-293 cells stably expressing recombinant human P2X7. Paroxetine inhibited dye uptake mediated by human P2X7 in a concentration-dependent manner with an IC₅₀ of 24 μ M and significantly reduces ATP-induced inward currents. We confirmed that trifluoperazine hydrochloride suppressed human P2X7 responses (IC₅₀ of 6.4 μ M). Both paroxetine and trifluoperazine did not inhibit rodent P2X7 responses and mutation of a known residue (P95L) did not alter the effect of either drug suggesting neither drug binds at this site. Finally, we demonstrate that P2X7-induced IL-1 β secretion from LPS-primed human CD14⁺ monocytes was suppressed with trifluoperazine and paroxetine.

Keywords

P2X7, antidepressant, paroxetine, fluoxetine, trifluoperazine, interleukin 1 β , ATP.

Non-standard abbreviations: SSRI selective serotonin reuptake inhibitor; IL-1 β interleukin 1
beta; LPS lipopolysaccharide

Introduction

The P2X7 receptor (P2X7) is a ligand-gated ion channel activated by extracellular ATP and is highly expressed on myeloid cells, including monocytes, macrophages, dendritic cells, and microglia, amongst other cell types including neurons and astrocytes [1]. Sustained activation of P2X7 can induce a variety of downstream signalling events and is a major physiological stimulus for the rapid secretion of pro-inflammatory cytokines of the interleukin-1 family including interleukin-1 β (IL-1 β) and interleukin-18 (IL-18) [2, 3]. Pro-inflammatory cytokines, including IL-1 β , induce sickness behaviour with depression-like symptoms in animals and an association between inflammation and depression is now well accepted [4-6]. Consequently, regulation of IL-1 β by the P2X7 receptor may be critical in the pathophysiology of depression and this could have important applications for P2X7 as a pharmacological target.

Genetic association studies have found some associations between P2X7 variants and mood disorders. A non-synonymous single nucleotide polymorphism (SNP) in *P2RX7*, rs2230912 encoding the amino acid change Q460>R, has been linked to major depression and bipolar disorder in some, but not all, genetic studies [7-13]. This SNP is inherited on a gain-of-function *P2RX7* haplotype and monocytes from healthy individuals carrying this gain-of-function haplotype displayed enhanced P2X7-mediated IL-1 β secretion [14]. In addition, it is known that the P2X7 knockout mouse has an anti-depressive phenotype [15, 16]. The expression of P2X7 in neurons is still debatable and therefore whether this is due to a reduced inflammatory capacity in central glial cells is currently unclear [17].

The aim of the present study was to investigate the effect of commonly prescribed psychoactive drugs on P2X7 responses *in vitro*. P2X7 receptors are known to bind a wide range of drug molecules displaying antagonism or potentiation of receptor responses (reviewed in [17,

18]). In addition to recently developed selective P2X7 antagonists, unrelated compounds such as KN-62 [19], calmidazolium [20], chelerythrine [21], a tyrosine kinase inhibitor [22], SB203580 [23], DIDS [24], clemastine [25], and probenecid [26] can also inhibit or potentiate P2X7 receptor responses. The related purinergic ion channel P2X4 is known to be blocked by several antidepressants with paroxetine, a selective serotonin reuptake inhibitor (SSRI), demonstrating the strongest inhibition [27]. The phenothiazine group of anti-psychotic agents including trifluoperazine have also recently been demonstrated to effectively block human P2X7 responses [28] however, it is not known if P2X7 responses are inhibited by other antidepressant and mood stabilising compounds. Some antidepressants have been demonstrated to have anti-inflammatory effects *in vitro* although their mechanism of action is unknown [4, 29]. Here we demonstrate that paroxetine is the only antidepressant to suppress recombinant human P2X7 responses and we investigated the anti-inflammatory efficacy of paroxetine and trifluoperazine in blocking IL-1 β secretion from human primary monocytes.

Methods

Materials

ATP, ethidium bromide, paroxetine maleate, paroxetine hydrochloride, fluoxetine hydrochloride, clomipramine hydrochloride, carbamazepine, lithium chloride, sodium valproate, and trifluoperazine hydrochloride were all from Sigma Aldrich (St. Louis, MO, USA). A-438079 hydrochloride and AZ11645373 were from Tocris Biosciences (Bristol, UK). Poly-D-lysine was from Merck Millipore (Billerica, MA, USA). Geneticin (G418) was from Life Technologies Australia.

Cell culture and transfection

HEK-293 cells were maintained in DMEM: F12 media (Life Technologies Australia) supplemented with 10% foetal bovine serum (Lonza Australia Pty Ltd, VIC, Australia), 100 U/ml penicillin, 100 µg/ml streptomycin and 5 mM L-glutamine (Life Technologies Australia). Stable cell lines expressing the human P2X7 receptor, rat P2X7 receptor and mouse P2X7 receptor were established by clonal dilution and kept under G418 selection media (Life Technologies Australia). Transient transfections were performed in 35 mm Petri dishes using 1 µg plasmid DNA and Lipofectamine 2000 (Life Technologies Australia) as per manufacturer's instructions.

Molecular biology

The wild-type human P2X7, rat P2X7 and mouse P2X7 constructs were obtained from Professors Annmarie Surprenant and Alan North, University of Manchester, UK. The human P2X7 gain-of-function variant termed P2X7-4 was used previously [14] and contains a combination of H155Y,

H270R, A348T and Q460R mutations. All constructs were verified by sequencing (SUPAMAC, University of Sydney, NSW, Australia).

Isolation of human monocytes

Peripheral venous blood was collected from genotyped healthy volunteers (both male and females) into heparin vacutainers (Becton Dickinson, NJ, USA). Written consent was obtained from each participant and the study was approved by the Nepean and Blue Mountains Local Health Network Human Ethics Committee Protocol No. 10/40-AU RED HREC/010/NEPEAN/89. All donors carried the gain-of-function SNP rs1718119 encoding the A348T mutation [14]. Mononuclear cells were isolated using Ficoll-Paque density centrifugation. CD14 positive monocytes were isolated by positive selection microbeads and MS columns (Miltenyi Biotech, Bergisch Gladbach, Germany).

Dye uptake experiments

Stably transfected HEK-293 cells were plated into alternate wells of poly-D-lysine coated 96 well plates (BD Falcon) at a density of 5×10^4 cells/well. The following day media was replaced with low divalent buffer (145 mM NaCl, 5 mM KCl, 13 mM D-glucose, 10 mM HEPES and 0.1 mM CaCl₂, pH 7.3) containing 25 μ M ethidium bromide. Cells were pre-incubated with drugs for 10 minutes at 37°C before the dye uptake assay was performed using a Fluostar Optima fluorescent plate reader (BMG Labtech GmbH, Ortenberg Germany) with excitation/emission filters 485nm and 510nm respectively. Gain settings were constant throughout each experiment. Basal fluorescence measurements were acquired for 40s followed by automatic injection of ATP (1 mM) and the kinetic measurement of fluorescence was performed for 4-10 minutes.

Electrophysiology

Whole-cell patch-clamp recordings were performed at room temperature using an EPC10 amplifier and Patchmaster acquisition software (HEKA, Lambrecht, Germany). Agonists and drugs were delivered using the RSC-160 fast-flow system (Bio-Logic Science Instruments, France). Membrane potential was clamped at -60 mV in all experiments. External solution was 145 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 13 mM D-glucose, 10 mM HEPES, and internal solution was 145 mM NaCl, 10 mM HEPES, 10 mM EGTA. Agonist was applied in a low divalent buffer as described for dye uptake experiments. All solutions were adjusted to pH 7.3 with 5M NaOH and were 295 - 310 mosm/L.

IL-1 β secretion assays

Purified CD14 positive human monocytes were plated at a cell density of 2×10^5 cells/well in a total volume of 250 μ l RPMI 1640 medium containing 1% FCS. Cells were primed with 100 ng/ml lipopolysaccharide (LPS) for 4 hours or medium alone (control) and were stimulated in duplicate. Drugs were added at the same time as LPS. ATP (3 mM) was added for 15 minutes and, following ATP stimulation, medium was removed from wells into clean eppendorf tubes. Supernatants were centrifuged briefly at 10,000 \times g to remove any non-adherent cells, and the resultant cell-free supernatants were stored at -80°C . A paired ELISA antibody set (BD Biosciences, San Jose, CA, USA) for human IL-1 β was used to detect IL-1 β in cell supernatants. Ninety-six-well plates were coated overnight with a capture anti-IL-1 β antibody, and a biotinylated anti-IL-1 β antibody was used for detection at the concentrations recommended by

the manufacturer. Recombinant human IL-1 β was used as standard over the concentration range 4 –250 pg/ml. Supernatants were diluted 1:50 in assay diluent (PBS containing 10% FCS).

Statistical Analysis

Data plotted are mean \pm SEM of three to four experiments. Graphs and statistical analysis was performed using GraphPad Prism version 6 (GraphPad Software Inc., La Jolla, CA, USA).

Statistical significance was assessed using unpaired t-tests or ANOVA with multiple comparison post-tests where appropriate. Significance was taken as $p < 0.05$ and is reported in figure legends.

Results

Our primary aim in this study was to determine whether psychoactive drugs for treatment of mood disorders such as depression and schizophrenia could affect human P2X7 responses *in vitro*. We screened HEK-293 cells stably expressing human P2X7 (HEK-hP2X7) in a dye uptake assay using ethidium bromide to measure the ATP-induced secondary permeability pathway. This has been used extensively to screen the effects of many compounds on P2X7 [18]. We tested three common antidepressants paroxetine, fluoxetine and clomipramine over the range 10 nM – 100 μ M. We found that paroxetine at concentrations >1 μ M could inhibit the ATP-induced dye uptake in HEK-hP2X7 cells (Figure 1). Further experiments revealed the IC₅₀ value for paroxetine on hP2X7-induced dye uptake to be 24 μ M (95% confidence interval 18.5–31.7 μ M, n=3 independent experiments). In comparison the IC₅₀ value for the selective P2X7 antagonist A-438079 was 0.5 μ M (95% confidence interval 0.41 – 0.61 μ M, n=3 experiments) (Figure 1B). This correlated well with other published data [30, 31]. In contrast a second SSRI fluoxetine, and a tricyclic antidepressant clomipramine, had no inhibitory effects on P2X7 responses. A small potentiation of ATP-induced dye uptake was seen with 30 μ M fluoxetine and significant potentiation was seen with 30 μ M clomipramine (Figure 1C, D).

In addition to antidepressants we tested several mood stabilising and psychoactive drugs for their effect on human P2X7 responses including lithium chloride (1 μ M – 1 mM), carbamazepine (0.1 μ M- 100 μ M), sodium valproate (10 μ M – 1mM) and trifluoperazine hydrochloride (100 nM – 100 μ M) (data not shown). Of these, only trifluoperazine hydrochloride (TFP) showed a significant inhibitory effect on human P2X7 receptors (Figure 2). In our hands the IC₅₀ value for inhibition of hP2X7-induced dye uptake by TFP was 6.4 μ M (95% confidence interval 5.6 – 7.3 μ M, n=3 experiments) which is slightly higher than previously reported [28].

Many compounds acting on human P2X7 have marked species selectivity [26, 32, 33]. We therefore investigated the effects of paroxetine, trifluoperazine, fluoxetine and clomipramine on recombinant rodent P2X7 receptors stably expressed in HEK-293 cells. Paroxetine and TFP showed no significant inhibitory effect on either rat P2X7 or mouse P2X7 (Figure 3). Similar to human P2X7, fluoxetine and clomipramine mildly potentiated the response at both rat and mouse P2X7 (Figure 3). Mean data is summarised in a bar chart (Figure 3C).

Previous work from Michel and colleagues have shown that a potential binding site for human specific P2X7 antagonists exists around amino acid residue 95 in the large ectodomain region of P2X7 [32]. We mutated the rat P2X7 at this position to the human equivalent (L>F) and determined whether this introduced sensitivity to paroxetine and TFP. We used AZ11645373 as a control since it has been demonstrated that this residue is involved in binding this human selective P2X7 antagonist [32]. We confirmed that wild-type rat P2X7 is not blocked by 100 μ M AZ11645373 but that the rat P2X7-95F mutant displays increased sensitivity to AZ11645373 (Figure 4). However, we found no increase in sensitivity to paroxetine or TFP in the rat P2X7-95F mutant (Figure 4).

Given the species selectivity of the paroxetine effect and its limitation to human P2X7 we decided to investigate whether the efficacy was different at a gain-of-function human P2X7 variant containing the Gln460>Arg mutation implicated in depressive disorders. We tested paroxetine and TFP on a P2X7-4.1 variant [14] expressed in HEK-293 cells and compared the level of inhibition to that of wild-type human P2X7. We found that paroxetine and TFP still inhibited responses at this gain-of-function variant and there was no significant difference in the degree of inhibition at 40 μ M paroxetine or 10 μ M TFP (Figure 5A).

To determine whether paroxetine binds to the human P2X7 receptor to block opening of the non-selective ion channel rather than having an effect on the secondary pore pathway directly, we performed patch-clamp experiments on HEK-hP2X7 cells. Inward currents were recorded in response to a five second pulse of 1 mM ATP in low divalent extracellular solution in the absence of paroxetine (30 μ M) and then following exposure to paroxetine for 5 minutes (Figure 5C). The ATP-induced inward current was significantly reduced to $28.9 \pm 5.9\%$ of control by 30 μ M paroxetine (n=4 cells) suggesting that paroxetine binds to and blocks the P2X7 receptor directly.

One of the most physiological, and likely pathophysiological consequences of P2X7 activation is the induction of IL-1 β secretion from monocytes, macrophages, and microglia. We determined whether the inhibition of proximal receptor signalling by paroxetine or TFP would translate to a reduction in cytokine secretion from freshly isolated human monocytes. We studied ATP-induced IL-1 β secretion from three donors. LPS (100 ng/ml) was used to prime human CD14⁺ monocytes to generate IL-1 β protein over a four hour incubation period and 3 mM ATP was used to induce cytokine secretion for a 15 minute period as previously described [14]. All drugs were added during the LPS priming step. The P2X7 antagonist, A-438079 (10 μ M) significantly blocked ATP-induced IL-1 β secretion by >95% in all donors (Figure 6). TFP (10 μ M) was also effective in blocking ATP-induced IL-1 β secretion by >95% in all donors. Paroxetine (30 μ M) reduced P2X7-mediated IL-1 β secretion to $39 \pm 14.3\%$ of the ATP control (Figure 6A) however this effect was not statistically significant until a higher concentration (100 μ M) of paroxetine was used. Fluoxetine (30 μ M) had no inhibitory effect in the ATP-induced IL-1 β secretion assay and enhanced P2X7-induced IL-1 β secretion to $148 \pm 33\%$ of the ATP control (Figure 6). To rule out an effect of paroxetine or trifluoperazine on protein synthesis of pro-IL-1 β during priming with LPS, we performed an additional set of experiments where we introduced

paroxetine, A438079 and trifluoperazine 15 minutes before the addition of ATP. Here we observed a similar complete inhibition of ATP-induced IL-1 β secretion by TFP and A438079 and a similar reduction in IL-1 β secretion by 30 μ M paroxetine ($45.5 \pm 17\%$ of control) which did not reach significance.

Discussion

P2X7 regulates the secretion of pro-inflammatory cytokines from monocytes and microglia, a process that is potentially linked to mood disorders [4]. The aim of our present study was to determine the effects of antidepressants and other commonly used psychoactive drugs on P2X7 responses and receptor mediated cytokine secretion. Previous studies have reported that several antidepressant compounds inhibit the related ATP-gated ion channel P2X4 [27], a receptor implicated in neuropathic pain and potentially also involved in inflammatory pathways [34-36]. We screened two common SSRIs paroxetine, and fluoxetine, and a tricyclic antidepressant clomipramine amongst several other psychoactive drugs. We chose this panel as they had produced the strongest effects on P2X4 responses in a recombinant expression system [27].

We show here for the first time that paroxetine but not fluoxetine or clomipramine inhibits human P2X7. In contrast to paroxetine, fluoxetine and clomipramine induced potentiation of P2X7-mediated dye uptake responses. Paroxetine and fluoxetine are both known to be potent inhibitors of the serotonin transporter IC₅₀ values of 1.1 nM and 25 nM respectively [37]. There are also well documented effects of antidepressants on multiple neurotransmitter receptors and ion channels including nicotinic acetylcholine receptors [38], 5-HT₃ receptors [39], voltage-gated Na⁺ [40], G-protein-activated inwardly rectifying K⁺ channels [41, 42], and hERG channels [43]. In addition, fluoxetine has been shown to potentiate GABA_A receptors through binding to an allosteric site on this ion channel [44]. Similar to other ion channels, P2X7 may have several allosteric sites capable of binding a broad range of chemical compounds that modulate their activity. The effects of antidepressants on P2X7 in this study were observed at higher concentrations than would be expected from the estimated therapeutic range for treating

clinical depression. However, these concentrations are in line with reports of block of other ion channels [38-41].

Comparing the effect of paroxetine on the two different P2X channels, the IC_{50} value for paroxetine on human P2X7 was an order of magnitude higher than that reported for human P2X4 [27]. We found that paroxetine did not have an inhibitory effect on recombinant rat P2X7 or mouse P2X7 receptors, therefore similar to a number of other antagonists, this drug demonstrates P2X7 species-selectivity [32, 45, 46]. This is also in contrast to the antidepressant effect on P2X4 where paroxetine inhibited both rodent and human P2X4 [27]. The differences in paroxetine IC_{50} and species selectivity between P2X7 and P2X4 receptors may reflect different modes of interaction between receptor and drug. It has been suggested that antidepressants may inhibit P2X4 by reducing surface expression of the channel through a lysosomal dependent process [47]. Our experiments suggest paroxetine may have a direct effect on P2X7, since acute application resulted in inhibition of ATP-induced inward currents. Furthermore P2X7 does not display desensitisation and internalisation of P2X7 through a lysosomal dependent pathway has not been demonstrated.

We found that the anti-psychotic agent trifluoperazine hydrochloride had a significant inhibitory effect on human P2X7 responses. Our data is in line with recent findings by Hempel et al, [28] who identified an inhibitory effect of phenothiazine class antipsychotics such as trifluoperazine as allosteric modulators of the human P2X7 receptor. Our calculated IC_{50} value for TFP on human P2X7 is slightly higher at 6.4 μM vs 1.8 μM [28]. We also found that TFP had no inhibitory effect at rodent P2X7 receptors. Furthermore mutation of L95 in rat P2X7 to the human P2X7 equivalent did not increase sensitivity to TFP suggesting TFP does not bind to

the same allosteric antagonist binding site as AZ11645373 [32]. A more extensive mutagenesis study will be required to determine the TFP and paroxetine binding site(s) on human P2X7.

P2X7 is an important regulator of IL-1 β secretion from monocytes, macrophages and microglia [2]. There is accumulating evidence of a link between inflammation and mood disorders [5, 6] with pro-inflammatory cytokines such as IL-1 β play a key role in triggering sickness behaviour and inflammation-associated depressive disorders [4, 5]. In this study we found that paroxetine reduced P2X7-mediated IL-1 β secretion from LPS-primed human monocytes from three donors. We have previously shown that the Q460>R mutation (rs2230912) is associated with a gain-of-function haplotype of *P2RX7* due to co-inheritance with a gain-of-function SNP (rs1718119) encoding an A348T mutation [14]. We found that paroxetine and TFP had the same inhibitory effect on the recombinant gain-of-function haplotype of human P2X7 (P2X7-4) compared to the wild-type recombinant human P2X7.

In this study we demonstrate two different drugs with different therapeutic uses having the same inhibitory effect on human P2X7. TFP has been previously demonstrated to inhibit acute inflammation [48] and chronic inflammatory pain [49], potentially through action on CaMKII as a molecular target. However, to the best of our knowledge, TFP is not known to exhibit a marked anti-depressive effect *in vivo*. Conversely, paroxetine has a known anti-depressant effect [37] and may work in some pain models [50] but little is known about its anti-inflammatory effects. Much evidence is available now for inflammatory-type reactions occurring in psychiatric patients [4, 5] but whether anti-inflammatory effects of selected therapeutic agents have any bearing on their clinical effect is not known.

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Figure Legends

Figure 1: The effect of antidepressants on recombinant human P2X7.

(a) Ethidium⁺ uptake was induced in HEK-hP2X7 expressing cells by the addition of 1 mM ATP (denoted by the arrow) in a low divalent physiological solution. Fluorescence was measured using a Fluostar Optima plate reader over a 300 second recording. Drugs were pre-incubated for 10 minutes at 37°C before ATP addition. Dye uptake in response to ATP is shown in black and in the presence of paroxetine (PAR) in red shapes. (b) Concentration response curve for inhibition of ATP-induced ethidium⁺ uptake by paroxetine (red) and the selective P2X7 antagonist, A-438079 (black). (c) Ethidium⁺ uptake to ATP in HEK-hP2X7 cells in the presence of 30 µM fluoxetine (FLUOX) (blue) or 30 µM clomipramine (CLOM) (green). (d) Bar chart demonstrating mild potentiation of ATP induced dye uptake by fluoxetine and significant potentiation by clomipramine (n=3 independent experiments). (e) Chemical structures of paroxetine, fluoxetine and clomipramine. * denotes $P < 0.05$ (ANOVA).

Figure 2: Trifluoperazine hydrochloride acts on human P2X7 responses.

(a) Ethidium⁺ uptake was induced in HEK-hP2X7 expressing cells by the addition of 1 mM ATP (denoted by the arrow) in a low divalent physiological solution. Fluorescence was measured using a Fluostar Optima plate reader over a 300 second recording. Trifluoperazine (10 µM) was pre-incubated for 10 minutes at 37°C before ATP addition and dye uptake in response to ATP is shown in black dots and in the presence of ATP and TFP in orange squares. (b) Concentration response curve for inhibition of ATP-induced ethidium⁺ uptake by TFP. Inhibition of P2X7 by 10 µM and 30 µM was significant ($p < 0.01$ by ANOVA with Dunnetts post-test, n=4 experiments) (c) Chemical structure of TFP.

Figure 3: Effect of antidepressants and TFP on rodent P2X7 responses.

(a) Ethidium⁺ uptake was induced in HEK-rP2X7 and (b) HEK-mP2X7 stable expressing cells by the addition of 1 mM ATP (denoted by the arrow) in a low divalent physiological solution. Fluorescence was measured using a Fluostar Optima plate reader over a 300 second recording timeframe. Drugs were pre-incubated for 10 minutes at 37°C before ATP addition. Control dye uptake response to ATP in the absence of drugs is shown in black. Dye uptake responses in the presence of paroxetine (PAR; red), fluoxetine (FLUOX; blue), clomipramine (CLOM; green) and TFP (orange). Mean data from three independent experiments is summarised in (c) using the same colour coding.

Figure 4: Residue 95 is not involved in paroxetine or TFP effect.

Ethidium⁺ uptake was measured in HEK-rP2X7 and HEK-rP2X7-95F mutant transiently transfected cells. The human specific P2X7 antagonist AZ11645373 (purple) does not inhibit wild-type rP2X7 however, mutation of L95 causes AZ11645373 to become effective at rP2X7. No inhibitory effect was seen for TFP or paroxetine in rP2X7-95F (lower panel). Representative uptake curves from two separate transfections are shown.

Figure 5: Paroxetine and TFP inhibit recombinant gain-of-function P2X7 variant to similar degree as wild-type P2X7.

(a) Bar chart showing percentage of control response to ATP in the presence of 10 μM PAR, 40 μM PAR, or 10 μM TFP for wild-type hP2X7 (black bars) and the P2X7-4.1 variant (white bars). * denotes p<0.05 and ** p<0.01 from ANOVA with Dunnetts post-test, n=3-4 experiments. (b) A representative ATP-induced ethidium uptake plot demonstrating that 40 μM

PAR (white squares) reduces the P2X7-4 variant response to ATP (black squares) to a level similar to that of WT hP2X7 response to 1mM ATP (black circles). C) Inward currents were recorded using whole cell patch clamp in HEK-hP2X7. Cells were voltage clamped at -60mV. Responses were elicited using 1mM ATP for 5 seconds before and after treatment with 30 μ M PAR (5 minutes). ATP was added in low divalent solution in the continued presence of PAR. Representative traces from three independent experiments are shown. Error bars represent standard error of the mean (S.E.M) * denotes $p < 0.05$ (unpaired t-test).

Figure 6: Paroxetine and TFP suppress P2X7-mediated IL-1 β secretion from human monocytes.

A) Purified CD14 positive monocytes (2×10^5 cells/well) from three donors were primed with 100 ng/ml LPS for four hours in RPMI media containing 1% FCS. 3 mM ATP was added for 15 minutes to elicit IL-1 β secretion. Supernatants were collected, frozen at -80°C and tested by ELISA. Monocytes were treated with drugs during LPS priming step, A-438079 (dark grey), paroxetine (PAR; red), and fluoxetine (FLUOX; green). Data is presented as % of ATP-induced secretion above LPS. Cells did not secrete IL-1 β in the absence of LPS (mean 36 pg/ml in media treated cells) and LPS did not induce an appreciable IL-1 β secretion (mean 420 pg/ml). 3 mM ATP elicited mean IL-1 β secretion of 4826 pg/ml (n=3 donors). * denotes $p < 0.05$ by one-way ANOVA with Dunnetts post-test. B) Purified CD14 monocytes from an additional three donors were treated with LPS (100ng/ml) for 4 hours. Drugs (A438079, PAR or TFP) were applied 15 minutes before the addition of ATP (3mM) for 15 minutes. Cells did not secrete IL-1 β in the absence of LPS (mean 38 pg/ml in media treated cells) and LPS did not induce an appreciable

IL-1 β secretion (mean 481 pg/ml). ATP elicited a mean IL-1 β secretion of 2863 pg/ml (n=3 donors) in these experiments. * denotes p<0.05 by one-way ANOVA with Dunnetts post-test.