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Vitamin B6 (Pyridoxal 5′ Phosphate) antagonises carotid body P2X3 receptors in hypertension

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Aims ATP acting on P2X3 receptors (P2X3R) within carotid bodies (CBs) underpins chemoreflex-mediated sympathetic overactivity in spontaneously hypertensive rats (SHR). Pyridoxal 5′ phosphate (PLP), the active form of vitamin B6, has been reported to act as a non-selective P2X receptor blocker. Hence, we hypothesised that PLP antagonism of P2X3R in the CB would treat hypertension.

Methods and results Herein, we employed a multipronged approach to investigate PLP's capability to attenuate CB hyperexcitability in hypertension.

First, PLP inhibited Ca^{2+} responses evoked by α, β-methylene ATP in cell lines expressing human (h) P2X3R with an IC50 of 8.7 μM. Next, *in-silico* data predicted that PLP binds to the same site of Gefapixant, supporting an allosteric antagonism. Using an isolated perfused carotid artery bifurcation-CB preparation, arterial infusion of PLP (50 μM; 15 min) attenuated CBs sensory firing in SHR ($P = 0.012$). Using the *in situ* working-heart brainstem preparation, carotid artery injections of PLP (1–5 mM) attenuated the chemoreflex-evoked sympathetic ($P = 0.023$) but not phrenic ($P = 0.62$) responses; the CB was stimulated with potassium cyanide (KCN,50 μL; 0.04%). In awake telemetered SHR ($n = 6$), intravenous infusion of PLP (48 mg/Kg/h; 30 min) attenuated KCN-evoked chemoreflex responses and reduced systolic, diastolic, and mean blood pressures (ΔMBP = −15.6 mmHg; $P = 0.025$). Translating our results, we performed a small double-blind, randomised clinical trial. In volunteers with hypertension ($n = 14$), oral supplementation with pyridoxine hydrochloride (600 mg) attenuated the hypoxic ventilatory response only in patients with high peripheral chemoreflex sensitivity ($P = 0.021$).

Conclusion Our findings suggest that PLP binds to and antagonises P2X3R and is a viable candidate for larger clinical trials to treat CB dysregulation in cardiovascular diseases.

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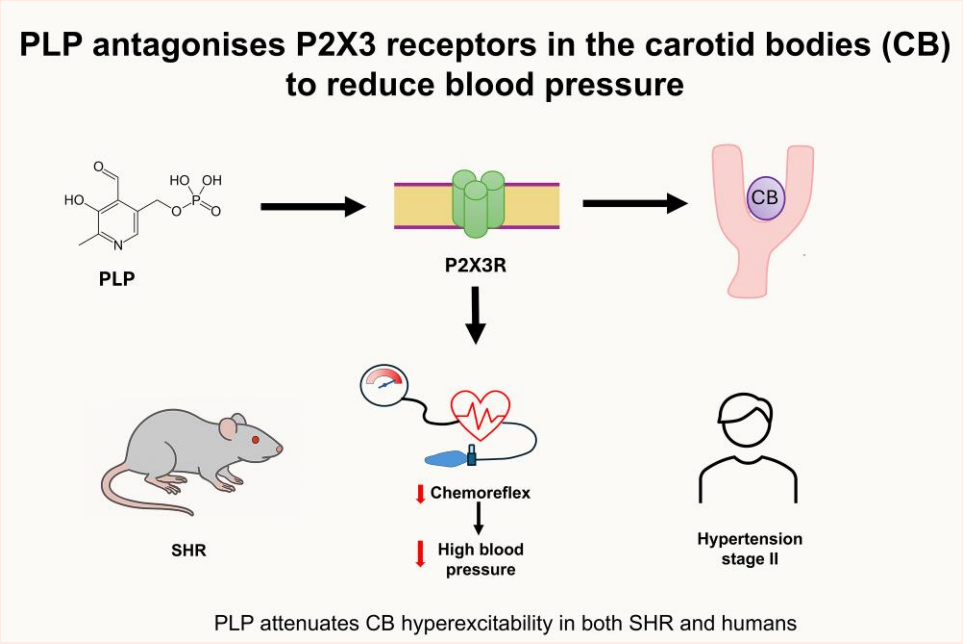
Graphical Abstract



Keywords Chemoreflex • Hypertension • P2X3 receptors • Carotid body • Vitamin B6 • Pyridoxal 5′ Phosphate

1. Introduction

Hypertension affects over 1 billion people worldwide and is the single most important predisposing risk factor for cardiovascular disease.[1] There is emerging evidence to suggest that a major contributor to hypertension and sudden cardiac death is an overactive sympathetic nervous system (i.e. fight or flight system) that is not well controlled by current medications.[2] Among the proposed causative mechanisms, aberrant carotid body (CB) discharge is linked to exaggerated sympathetic nerve activity (SNA) generation.[3]

The CBs are the main peripheral chemoreceptors in the body and are responsible for monitoring the arterial blood milieu including arterial partial pressures of oxygen (PaO_2) and carbon dioxide ($PaCO_2$), and pH.[4] CB activation results in increased ventilation and SNA, leading to increases in heart rate, cardiac contractility, vasoconstriction, and ultimately blood pressure.[5] Clinical trials in which one CB was surgically resected in patients with drug-resistant hypertension validate it for the first time as an effective/potent therapeutic target.[6,7] However, the nature of this intervention was associated with adverse side effects.[6,8] Therefore, an ideal approach would be to reduce CB sensitivity pharmacologically to physiological levels.

Pre-clinical animal work established a critical role of adenosine 5′ triphosphate (ATP) as a transmitter for the CB in the transduction of hypoxia.[9,10] In spontaneously hypertensive rats (SHR), upregulation of P2X3 subunit receptors (P2X3R) underpins CB hypertonicity and hyperreflexia.[11] Moreover, targeting P2X3R *in vivo* with a highly potent antagonist, such as MK-7264 (i.e. Gefapixant) demonstrated promising results in both hypertension and heart failure.[11,12] Such an approach awaits clinical trials in human patients as P2X3R antagonists are already employed for other indications.[13,14]

Pyridoxal 5′ phosphate (PLP), the active form of vitamin B6 (pyridoxine hydrochloride; PHC), was previously suggested to act as a non-selective P2X receptor blocker.[15] Moreover, *low* PLP levels in the blood have been associated with increased risk of cardiovascular disease and all-cause mortality in hypertensive patients.[16,17] Rats placed on a PHC-deficient diet for 8 weeks developed sympathetic-mediated hypertension.[16] In humans, oral supplementation with PHC reduced blood pressure and circulating catecholamines in hypertensive patients[18]; however, in this study there was no placebo group. Hence, we hypothesised that PLP antagonism of P2X3R in the CB would lower blood pressure in hypertension.

Herein we employed *in silico*, *in vitro*, and *in situ* approaches, along with *in vivo* animal models, to investigate the capability of PLP to attenuate CB hyperexcitability in hypertension. We also carried out a small double-blind randomized clinical trial to investigate PLP action on peripheral chemoreflex sensitivity in participants with hypertension. Our results suggest that PLP is a viable candidate for a larger clinical trial to treat CB dysregulation in cardiovascular-respiratory diseases.

2. Materials & methods

Detailed description of *Materials & Methods* and *Additional Results*, including *Statistical Output*, is provided in the Supplemental material.

2.1 Ethical approval

A total of thirteen male Wistar rats and forty-two male spontaneously hypertensive rats (SHRs) were bred by the Vernon Jansen Unit (VJU) of the University of Auckland. All tests were performed in accordance with the biomedical research guidelines for animal welfare and were approved by the University of Auckland committee for the ethical use of animals in scientific research (AEC# 2274, #22280, and #25105). All animal procedures performed were in accordance with the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes.

Ethical approval for in-human study was provided by the Northern A Health and Disability Ethics Committee, Auckland, New Zealand (20/MTA/29), by the Auckland District Health Board Research Review Committee (A + 9025) and was registered with the Australian New Zealand Clinical Trials Registry (URL: https://www.anzctr.org.au/; identifier:

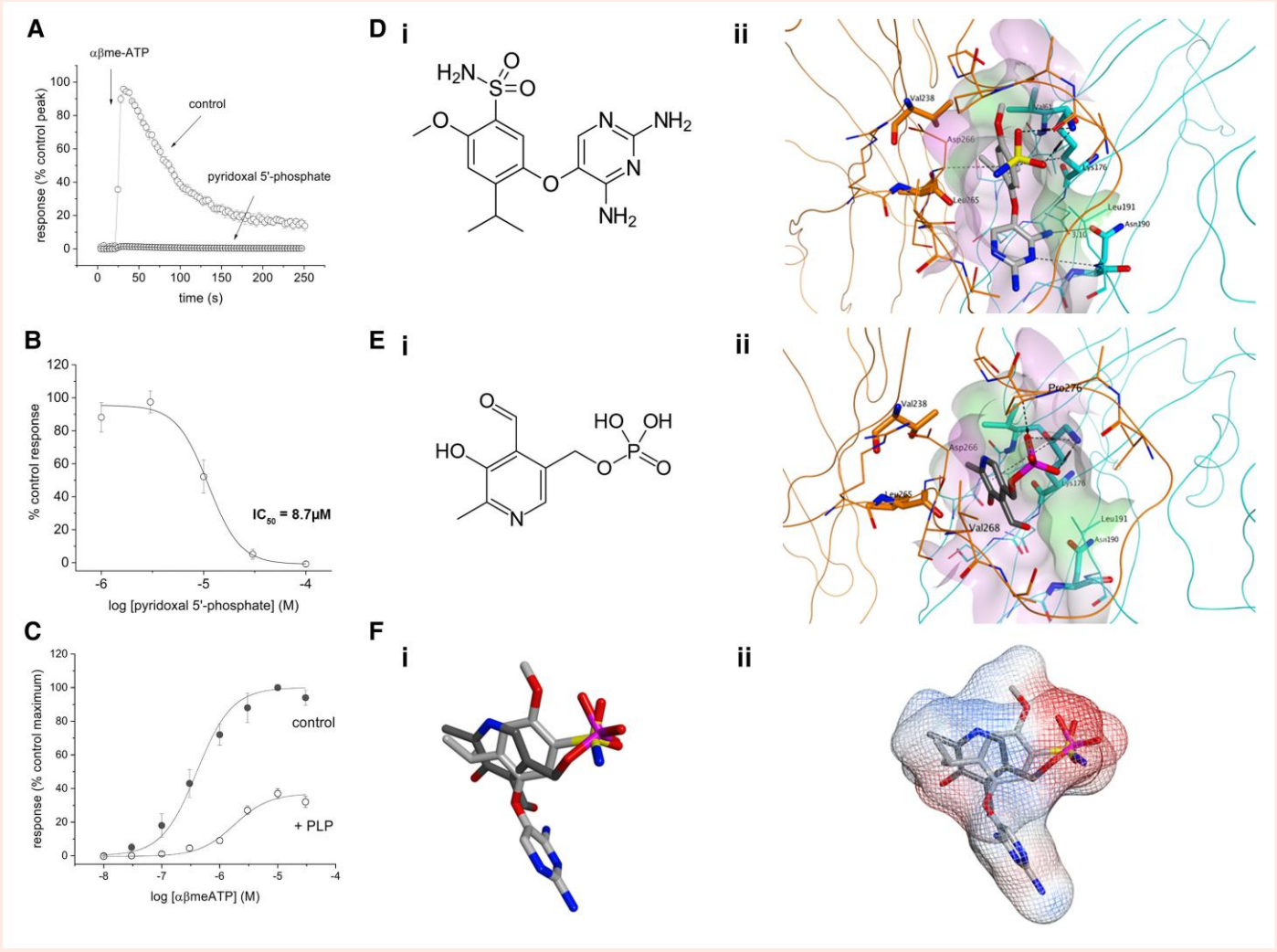


Figure 1 Pyridoxal 5′ phosphate (PLP) vs. MK-7264 binding to human P2X2/3 receptor (hP2X3R). *A*) In cell lines ($n = 5$ plates; 25 000 cells/well, run in duplicate) expressing hP2X3R, PLP inhibits Ca^{2+} responses evoked by α, β-methylene ATP (αβme-ATP, 10 μM). *B*) The dose-response curve indicates an IC_{50} of 8.7 μM for PLP. *C*) In pharmacodynamic studies, PLP reduced both the potency and efficacy of αβme-ATP on hP2X3R, revealing an allosteric mode of antagonism ($n = 3$ plates each—control vs. PLP; 25 000 cells/well, run in duplicate). *D*) i: Molecular structure of MK-7264. ii: Binding of MK-7264 (carbon atoms in light grey) to the allosteric antagonist site of hP2X3R (PDB ID 5YVE), between the extracellular domains of two subunits (LB from one subunit, LF and LB from the adjacent subunit) (ribbon and carbon atoms in orange and light blue, respectively; third subunit hidden for clarity). The receptor molecular surface defining the site is represented according to its lipophilic/hydrophilic nature (green: lipophilic, pink: hydrophilic, white: neutral). *E*) i: Molecular structure of PLP. ii: Predicted binding pose of PLP (carbon atoms in dark grey) to the allosteric antagonist site in the hP2X3R crystal structure (PDB ID 5YVE). The receptor molecular surface defining the site is represented according to its lipophilic/hydrophilic nature (green: lipophilic, pink: hydrophilic, white: neutral). *F*) Superposition between the structure of MK-7264 co-crystallized with hP2X3 (PDB ID 5YVE, carbon atoms in light grey) and the predicted docking pose of PLP to the same site of the 5YVE crystal structure (carbon atoms in dark grey). i: ligands represented as simple sticks. ii: the ligand electrostatic surface shown for both ligands (blue: positive (partial) charge, red: negative (partial) charge, white: neutral). Source: Wikipedia: https://pt.wikipedia.org/wiki/Piridoxal_fosfato and https://en.wikipedia.org/wiki/Gefapixant

ACTRN12620001121954). All participants were provided with a comprehensive written and verbal explanation of the study protocols and provided written informed consent prior to participation. The study was conducted according to the Declaration of Helsinki (2013).

2.2 Study design

Six experiments were performed to investigate whether PLP (*Figure 1E, I*) is a suitable candidate for treating CB-mediated hypersensitivity in cardiovascular disease. First, in 1321N1 cells stably expressing hP2X2/3R, we investigated whether PLP could inhibit Ca^{2+} responses evoked by α, β-methylene ATP *in vitro*. Next, using a crystal structure of the hP2X3 receptor in complex with the negative allosteric modulator MK-7264 (PDB ID 5YVE19), we investigated how PLP may interact on the same negative allosteric binding site as MK-7264 (*Figure 1D, I*) via a molecular docking analysis and molecular dynamic simulations. Third, we tested PLP's ability to attenuate CB excitability as measured via carotid sinus nerve (CSN) recordings *in vitro*. Fourth, using an *in situ* preparation, we investigated the effect of PLP on the peripheral chemoreflex motor responses. Fifth, in adult SHRs telemetered for arterial pressure measurement, an intravenous infusion of PLP was carried out to test its ability to both attenuate the peripheral chemoreflex response and lower blood pressure *in vivo*. We also quantified the level of gene expression in the CB of SHR relative to Wistar rats for the enzymes responsible for the breakdown of PLP. Finally, we carried out a small double-blind, randomised clinical trial to test whether PLP would attenuate peripheral chemoreflex sensitivity in patients with hypertension.

2.3 *In vitro* studies

A 1321N1 cell line stably expressing the human P2X2/3 receptor[19] was maintained in Dulbecco's Modified Eagle Medium containing 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 50 U/mL penicillin, 50 µg/mL streptomycin, and 250 ng/mL puromycin. Cells were loaded with Fura-2 for intracellular Ca^{2+} assays. Detailed description of *Materials & Methods* is provided in Supplemental material.

2.4 In silico studies

Molecular modelling experiments were performed on an Asus WS X299 PRO Intel® i9-10980XE CPU @ 3.00 GHz × 36 running Ubuntu 18.04 (graphic card: GeForce RTX 2080 Ti). Molecular Operating Environment (MOE, 2022.02, Montreal, QC, Canada) and Maestro (Schrödinger Release 2024-4, New York, NY, USA) were used as molecular modelling software. Detailed descriptions of molecular docking and molecular dynamics simulations are provided in the Supplemental material.

2.5 In vitro CSN recording

Four- to five-week-old ($n = 8$) and six- to eight-week-old ($n = 6$) male SHR were deeply anaesthetized with 5% isoflurane in O_2 (1 $L.min^{-1}$) and euthanised by exsanguination. The left and right intact carotid artery bifurcations containing the CSN and the CB were exposed and harvested via a longitudinal incision on the ventral surface of the neck. The bifurcation was surgically resected and subsequently placed in a recording chamber and superfused continuously with Ringer's solution (composition in mmol/L as follows: NaCl, 125; NaHCO3, 24; KCl, 3.75; CaCl2, 2.5; MgSO4, 1.25; KH2PO4 1.25, and D-glucose 10; Sigma-Aldrich). Sensory activity from the CSN was recorded from the cut end of the nerve using a glass suction electrode. Detailed description of *Materials & Methods*, including experimental protocols, is provided in Supplemental material.

2.6 In situ working heart-brainstem preparation (WHBP)

Juvenile SHRs ($n = 15$, 3–6 weeks old, 50–90 g) were anaesthetised deeply with isoflurane (5% in O_2, 1L min^{-1}, via inhalation) until loss of paw and tail withdrawal reflexes and then heparinized (350 UI i.p.; Pfizer). Subsequently, animals were euthanized via exsanguination following bisection below the diaphragm. After cooling the upper body in Ringer's solution (composition in mmol/L as follows: NaCl, 125; NaHCO3, 24; KCl, 3.75; CaCl2, 2.5; MgSO4, 1.25; KH2PO4 1.25; and D-glucose 10; Sigma-Aldrich), animals were decerebrated pre-collicularly, the lungs were removed, and the descending aorta isolated and cannulated with a double-lumen catheter for perfusion. Detailed description of *Materials & Methods*, including experimental protocols, is provided in Supplemental material.

2.7 In vivo blood pressure telemetry

Under anaesthesia with isoflurane (2–5% in O_2, 1 L min^{-1}, via inhalation), adult male Wistar and SHRs ($n = 5$ each, 30–34 weeks old, 300–350 g) were given a single abdominal subcutaneous injection of analgesic (0.05 mg/Kg of Vetergesic—buprenorphine), anti-inflammatory (2 mg/Kg of Meloxicam—Metacam), local anaesthetic (6 mg/Kg Bupivacaine 0.25%, 2.5 mg/mL+/−1:400 000 epinephrine -Marcaine), and antibiotic (4 mg/Kg of Baytril—enrofloxacin). Under aseptic technique, a midline abdominal incision of 3–4 cm was made, and the catheter of the blood pressure telemeter (either HD-S10, DSI, or TRM54P, Kaha Science -AD Instruments) was implanted into the abdominal aorta. During the same surgery, an intravenous line was inserted into the right femoral vein for intravenous drug administration. After the surgery, analgesic (0.05 mg/Kg—buprenorphine) and anti-inflammatory (2 mg/Kg of Meloxicam—Metacam) were given subcutaneously once a day for a minimum of 3 days, and the femoral line was flushed with heparinised saline solution every 2 days throughout the time of experiments. At the end of experiments, animals were euthanised via intravenous injection of Pentobarb 300 (800 mg/kg—Sodium Pentobarbitone—Provet NZ Pty Ltd, New Zealand). Detailed description of *Materials & Methods*, including experimental protocols, is provided in Supplemental material.

2.8 Barometric whole-body plethysmography

Animals were placed inside a custom-made chamber with controlled air inflow and outflow. Tidal volume (V_T) was calculated using the barometric method of Drorbaugh & Fenn.[20] The respiratory frequency (fR) was derived from pressure oscillations, and the minute ventilation (V_E) calculated as the product of f_R and V_T. Detailed description of *Materials & Methods*, including experimental protocols, is provided in Supplemental material.

2.9 RT-qPCR for ALP gene expression

Steady-state gene expression analysis in the CBs was performed on $n = 8$ male, 4–6 weeks old Wistar and SHR rats. Rats were anaesthetised deeply with isoflurane (5% in O_2, 1L min^{-1}, via inhalation) until loss of paw and tail withdrawal reflexes; subsequently, animals were euthanised via exsanguination, and CBs were micro-dissected from carotid artery bifurcation and snap-frozen in liquid nitrogen. Samples were stored at −80°C until batch processed. Detailed description of Materials & Methods for RNA extraction and processing is provided in Supplemental material.

RT-qPCR was carried out in triplicates using Luna® Universal qPCR Master Mix (Cat. # M3003; New England Biolabs) on a QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems, USA). Eukaryotic translation initiation factor 4B (Eif4b; ENSRNOG00000010103) was used as a housekeeping control as described previously.[20] Detailed description of primers is provided in Supplemental material.

2.10 Double-blind randomised clinical trial

We conducted a double-blind randomised placebo-controlled crossover study to test whether PHC would attenuate the chemoreflex sensitivity in patients with hypertension. Participants attended the laboratory at the University of Auckland Clinical Research Centre on three separate occasions for a familiarization visit and two experimental visits (see Supplemental material online, *CONSORT 2025 flow diagram*).

Participants were asked to abstain from caffeine for 12 h prior, alcohol and exercise after 2000h the evening before the study, and any 'over the counter' (e.g. paracetamol) or cardioactive medications (beta-blocker, ACE inhibitor, angiotensin receptor blockers, etc.). On the morning of the experimental visits, participants received either placebo or vitamin B6 (600 mg PHC in 24 mL liquid). After 2 h, participants were instrumented for measurements of cardiorespiratory variables while the hypoxic ventilatory response (HVR) was evoked using an isocapnic hypoxic rebreathing protocol. Thus, our primary outcome is the HVR, while our secondary outcomes include blood sample analysis and resting cardiorespiratory variables. Detailed description of *Materials & Methods*, including experimental protocols, is provided in Supplemental material.

2.11 Statistical analysis

Graphic and statistical analyses were performed using GraphPad Prism (version 9.3.1, USA) and Jamovi (Version 2.4.8; retrieved from https://www.jamovi.org). Paired and unpaired Student's *t*-test, and mixed regression models were used accordingly (For details, see *detailed statistical analysis per dataset*). In our analysis, we fitted the data in 2–3 different models to streamline which one best described it. The criteria to choose the final model were based, first, on the analysis of residuals and, second, on the value of Akaike Information Criterion (AIC) goodness of fit. As a general approach, we first fitted our data using a linear model assuming normal distribution. However, if the analysis of residuals exposed a violation of the assumption of normality and/or heteroskedasticity of residuals, then we would fit our data using a generalized gamma distribution model with link function identity, which can accommodate a variety of data with

skewed continuous distribution profile. The level of significance was set at 5%, and data were expressed as mean ± standard deviation (SD).

3. Results

3.1 PLP allosterically inhibits recombinant hP2X3R activity *in vitro*

PLP inhibited α, β-methylene ATP-evoked Ca^{2+} responses in 1321N1 cells stably expressing human P2X2/3R (*Figure 1A*) with an IC_{50} of 8.7 ± 0.7 μM ($N = 5$; *Figure 1A* and *B*). Furthermore, PLP significantly increased the α, β-methylene EC_{50} from 433 ± 92 nM to 1706 ± 189 nM ($N = 5$; $P < 0.01$) (i.e. right-shift, reducing its potency) and reduced the maximal response to α, β-methylene by 63% ($N = 5$; $P < 0.01$) (i.e. reducing its efficacy), suggesting an allosteric mode of antagonism (*Figure 1C*). We have previously characterized the action of the allosteric antagonist MK-7264 at human P2X3 and P2X2/3 receptors,[21] which is proposed to exert action through binding an allosteric site created by contacts in the left flipper, lower body, and dorsal fin receptor domains.[22]

3.2 *In silico* analysis predicts less strong PLP binding to hP2X3's negative allosteric site

The predicted binding of PLP to the same pocket as MK-7264 in the crystal structure of its complex with hP2X3 (PDB ID 5YVE) was evaluated via molecular docking and supports its antagonistic activity. In the crystal structure of its complex with hP2X3R (*Figure 1D*), MK-7264 engages with residues defining the site through a network of interactions: a hydrogen bond/electrostatic interaction between the sulphonamide oxygen atoms and the sidechain of Lys176; a hydrogen bond between the pyrimidine ring *N*-2 position and the backbone NH group of Leu191, and two hydrophobic interactions between the phenyl ring and the carbon atoms of Asp266 (backbone C-α) and Lys176 (sidechain C-β). In addition, the amine group at position 4 of the pyrimidine ring is at H-bond distance from the carbonyl oxygen of the sidechain of Asn190.

Following a molecular docking analysis performed with GlideXP,[23] PLP is predicted to bind to the upper portion of the pocket defined by MK-7264 (*Figure 1E*), with its phosphate group overlapping the sulphonamide function of MK-7264 (*Figure 1F, I*) and its pyridine ring mainly occupying the space defined by the phenyl ring of MK-7264 (*Figure 1F, I*). PLP is predicted to make an ionic bond and a hydrogen bond, through its phosphate group, with the sidechain of Lys176. One oxygen atom in the phosphate group is also predicted to form a weaker interaction with the C-α of Pro276. The pyridine ring appears to retain the ability to form a hydrophobic interaction with the sidechain C-β of Lys176, while the methyl group is near the sidechain of Val238, with the potential for an additional hydrophobic interaction. Next, we performed triplicate, 100 ns molecular dynamic simulations of MK-7264 and PLP in complex with hP2X3R, to compare their predicted binding energies to the negative allosteric site. MK-7264 optimized its occupation of the site, maintaining a stable position during the entire simulation. The compound adjusted its orientation in the binding pocket, establishing a network of polar interactions with the residues defining the binding site (i.e. Lys176, Asn190, Leu191, Asp266, Ser267, and Gly277), as summarised in Supplementary material online, *Figure S5*. In the opposite direction, PLP did not assume a stable conformation in the binding pocket, showing variable results in terms of occupation of the binding site across the three simulations performed, consistently moving towards, and protruding from, the upper part of the allosteric pocket. Although the protein-ligand complex reached stability after about 20 ns, as observed for MK-7264, highly variable binding modes were obtained for this compound, suggesting weaker binding to this site compared to MK-7264. This was supported by the predicted binding energies ($\Delta G_{binding}$), evaluated using the Prime/MM-GBSA calculation method,[24] of −52.6 ± 4.9 KJ/mol ($N = 3$) and −22.7 ± 9.7 KJ/mol ($N = 3$) for MK-7264 and PLP, respectively.

3.3 PLP infusion suppresses carotid sinus nerve (CSN) firing in SHR

To test functionally whether PLP was capable of antagonising P2X3R in the peripheral chemoreceptors of SHR, we tested its ability to attenuate CB hypertonicity and hyperreflexia[11] via CSN recordings. First, as proof-of-principle, PLP was bolus perfused at a high dose (5 mM; 5 mL) to test whether it would block potassium cyanide-evoked CSN responses (*Figure 2A*; KCN, 100 μL, 0.08%); the perfusate was gas-equilibrated with carbogen (5% CO_2 in 95% O_2, $PO_2 > 400$ mmHg). PLP strongly suppressed CSN firing evoked by KCN injection [*Figure 2A*; ΔCSN = −0.86 μV (95% CI, −1.07 to −0.66); $P < 0.001$, Supplementary material online, *Table S5*]. In a few instances, the evoked sensory discharge was completely abolished (*Figure 2A*). Interestingly, 5 min after the infusion was finished, the effect began to wane [ΔCSN = −0.74 μV (95% CI, −0.95 to −0.53); $P < 0.001$, Supplementary material online, *Table S5*]. Next, in normoxia (i.e. perfusate PO_2= 90–100 mmHg), continuously intra-arterial infusion of PLP (50 μM; 15 min) significantly attenuated ongoing CBs sensory firing [*Figure 2B*; ΔCSN = −11.44 impulse/s (95% CI, −20.8 to −2.11); $P = 0.022$, Supplementary material online, *Table S6*, *Figure 2B]*, indicating its ability to also reduce tonicity, which has been previously demonstrated in SHR.[11]

3.4 Intra-arterial injections of PLP attenuate the CB-evoked sympathetic reflex *in situ*

To determine the effect of PLP on the peripheral chemoreflex motor responses, we challenged the CB with KCN whilst recording thoracic sympathetic chain activity (tSNA), phrenic nerve (PN), and ECG (i.e. heart rate—HR) in the *in situ* working heart-brainstem preparation—WHBP.[25,26] Focal injections of PLP (1–5 mM) into the internal carotid artery significantly attenuated the CB-evoked sympathetic reflex [$\Delta tSNA_{PLP} = -0.12$ μV vs. control KCN (95% CI, −0.23 to −0.019); $P = 0.023$, Supplementary material online, *Table S7*, *Figure 2C]*. We also observed an attenuating trend in the CB-evoked bradycardia (i.e. $P = 0.056$, Supplementary material online, *Table S8*, *Figure S6A*), whereas no effect was observed on either chemoreflex-evoked PN rate or PN amplitude (i.e. $P > 0.05$, Supplementary material online, *Tables S10* and *S11*). However, PLP significantly attenuated the KCN-evoked increase in neural inspiratory drive [i.e. PN amplitude/inspiratory time −Δ PN amp/Ti = −0.67 μV/s vs. control KCN (95% CI, −1.05 to −0.304); $P = 0.023$, Supplementary material online, *Table S9*, *Figure S6D*].

3.5 PLP infusion attenuated the KCN-evoked pressor response in telemetered conscious SHR

To test whether PLP would reproduce the above-mentioned results *in vivo*, we evoked chemoreflex responses with bolus injections of KCN −1 μg/μL; 15 μg[27] in adult telemetered SHR ($n = 5$) and 30 μg in Wistar rats ($n = 5$). Ventilation was recorded via the barometric whole-body Plethysmography.[19] In SHRs, KCN-evoked pressor response was attenuated during PLP infusion (24 mg/Kg) when compared to control KCN injection [ΔMBP = −48.3 mmHg vs. control KCN (95% CI, −73.3 to −23.34), $P = 0.002$, Supplementary material online, *Table S14*, *Figure* 3_{A3}]; whilst neither bradycardia [$\Delta HR = 46$ bpm vs. control KCN (95% CI, −97 to 188), $P = 0.48$, Supplementary material online, *Table S16*, *Figure* 3_{A4}] nor tachypnoea were significantly affected [$\Delta f_R = -86$ breaths/min vs. control KCN (95% CI, −180 to 9), $P = 0.07$, Supplementary material online, *Table S15*, *Figure* 3_{A5}]. Likewise, no effect was observed on tidal volume (V_T) [$\Delta V_T = -0.1$ mL/Kg vs. control KCN (95% CI, −0.9 to 0.7), $P = 0.82$, Supplementary material online, *Table S17*, *Figure* 3_{A5}], minute ventilation (V_E) [$\Delta V_E = -150$ mL/min/Kg vs. control KCN (95% CI, −360 to 59), $P = 0.137$, Supplementary material online, *Table S18*, *Figure* 3_{A5}], or respiratory efficiency (V_E/VCO_2) [$\Delta V_E/VCO_2 = -3.27$ vs. control KCN (95% CI, −8.2 to 1.7), $P = 0.168$, Supplementary material online, *Table S19*, *Figure* 3_{A5}].

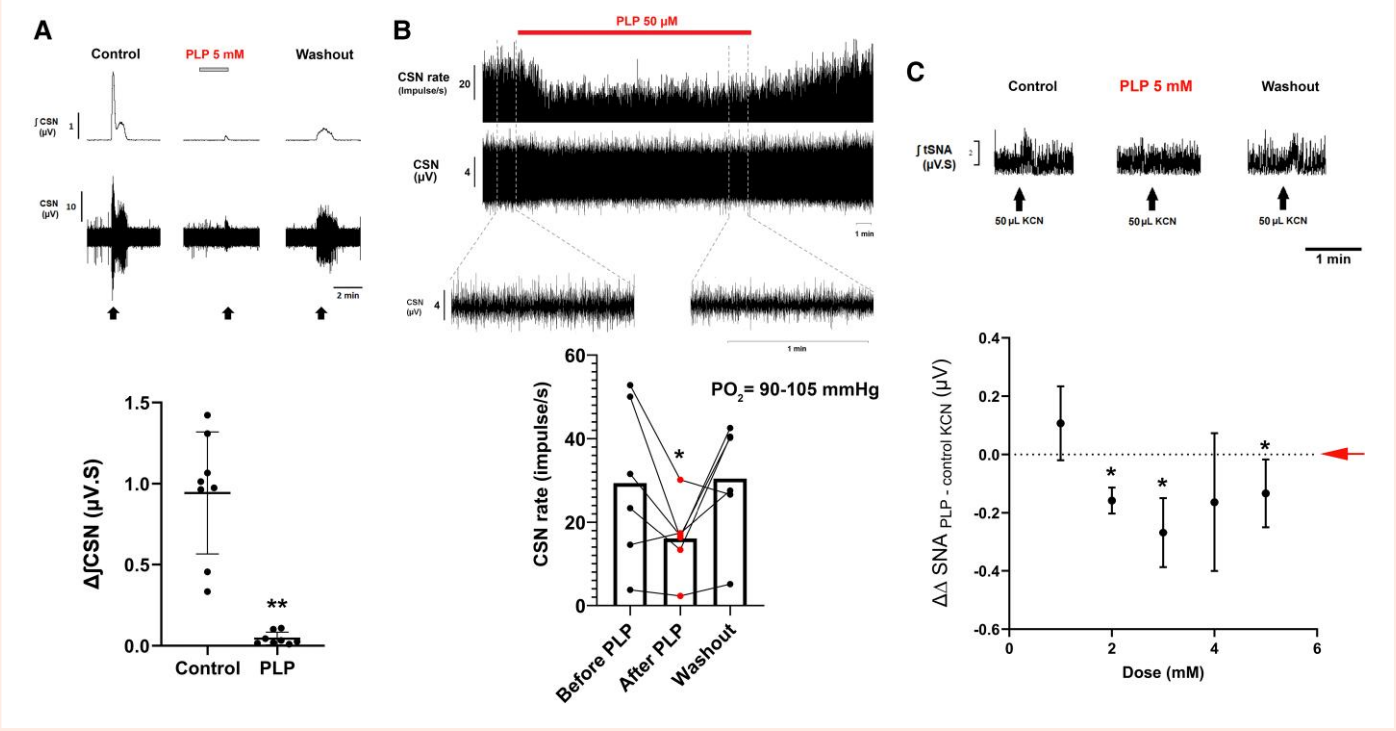


Figure 2 Effect of pyridoxal 5′ phosphate (PLP) on carotid body (CB) activity *in vitro* and *in situ*. *A*) In the isolated perfused carotid artery bifurcation-CB preparation of pre-hypertensive SHR (4–6 weeks, $n = 8$ rats), we used potassium cyanide (KCN; vertical arrow—100 μL, 0.08%) to evoke carotid sinus nerve (CSN) responses either in the presence or absence of PLP (5 mL, 5 mM) (i.e. assessment of hyperreflexia). At the top is a typical tracing of raw (CSN) and integrated sinus nerve discharge (ʃCSN). *B*) Under normoxia ($PO_2 = 90–105$ mmHg), PLP (50 μM, 15 min) was continuously infused through the bifurcation to record its effect on resting CSN firing (SHR, 4–6 weeks, $n = 6$ rats). At the top is a typical tracing of raw CSN and its discharge rate. *C*) In the *in situ* working heart-brainstem preparation, focal injections of PLP (1–5 mM) into the internal carotid artery attenuated the CB-evoked sympathetic chemoreflex (SHR, 4–6 weeks, $n = 15$ rats). At the top is a typical tracing of integrated thoracic sympathetic chain activity (ʃtSNA); data are shown as ΔΔ, which means the difference between the ΔʃtSNA responses from PLP vs. the first KCN (i.e. control response). The further the data departs from the dashed line at zero (horizontal arrow), the more attenuated the response. Data were analysed using a mixed regression model with either normal or gamma distribution. Mean ± SD, * $P < 0.05$, **$P < 0.01$.

Likewise, in Wistar rats, KCN-evoked cardiovascular responses were attenuated during PLP infusion—both pressor [ΔMBP = −24.1 mmHg vs. control KCN (95% CI, −40.3 to −7.84), $P = 0.011$, Supplementary material online, *Table S20*, *Figure S7A*] and bradycardic responses [Δ*HR* = 96.4 bpm vs. control KCN (95% CI, 26 to 166), $P = 0.015$, Supplementary material online, *Table S21*, *Figure S7B*]. On the other hand, neither tachypnoea [ΔfR = 28.9 breaths/min vs. control KCN (95% CI, −52 to 109), $P = 0.442$, Supplementary material online, *Table S22*, *Figure S7C*] nor ventilation [ΔVE = −160 mL/min/Kg vs. control KCN (95% CI, −353 to 32), $P = 0.101$, Supplementary material online, *Table S23*, *Figure S7E*] was significantly affected.

3.6 Intravenous infusion of PLP lowers blood pressure and CO_2 production (VCO_2) of SHR

We evaluated PLP infusion on resting blood pressure, breathing, and metabolism of both Wistar and SHRs ($n = 5$ each) (see Supplementary material online, *Tables S1* and *S2*). In SHRs, PLP prompted significant falls in systolic [ΔSBP = −17 mmHg (95% CI, −29 to −5); $P = 0.019$, Supplementary material online, *Table S25*, *Figure* 4_{A1}], diastolic [ΔDBP = −16 mmHg (95% CI, −22.4 to −9.3); $P = 0.003$, Supplementary material online, *Table S26*, *Figure* 4_{A2}], mean blood pressures [ΔMBP = −16.2 mmHg (95% CI, −24.3 to −8.0); $P = 0.005$, Supplementary material online, *Table S27*, *Figure* 4_{A3}], and heart rate [ΔHR = −35 bpm (95% CI, −57.6 to −12.7); $P = 0.012$, Supplementary material online, *Table S28*, *Figure* 4_{A4}]. PLP did not produce significant changes in breathing ($P > 0.05$; Supplementary material online, *Tables S29–S33*) (see Supplementary material online, *Table S1*). Regarding metabolism, PLP reduced CO_2 production [$\Delta VCO_2 = -8.53$ mL/min/Kg (95% CI, −14.1 to −2.98); $P = 0.020$, Supplementary material online, *Table S35*, *Table S1*] without a change in oxygen consumption [$\Delta VO_2 = -2$ mL/min/Kg (95% CI, −9.1 to 4.78); $P = 0.490$, Supplementary material online, *Table S34*], which reduced the respiratory exchange ratio [ΔRER = −0.22 (95% CI, −0.42 to −0.02); $P = 0.054$, Supplementary material online, *Table S36*], (see Supplementary material online, *Table S1*).

In Wistar rats, PLP also prompted significant falls in blood pressure (see Supplementary material online, *Table S2*); however, the latter was only a fraction of what we have seen in SHRs (ΔSBP = −4.6 mmHg (95% CI, −8.5 to −0.8); $P = 0.042$, Supplementary material online, *Table S38*; ΔDBP = −3.5 mmHg (95% CI, −6.3 to −0.8); $P = 0.033$, Supplementary material online, *Table S39*; ΔMBP = −4.0 mmHg (95% CI, −7.2 to −0.9); $P = 0.035$, Supplementary material online, *Table S40*). PLP did not produce significant changes in HR and breathing ($P > 0.05$; Supplementary material online, *Table S41–S49*) (see Supplementary material online, *Table S2*).

3.7 The CBs of SHR display elevated gene expression for alkaline phosphatase (ALP) subtype *Alpl*

Given that the effects of PLP were short lived, we analysed the gene expression of alkaline phosphatases (ALP), which is one of its main degrading enzymes. Based on published RNA-seq data,[20] the CB of SHR displays increased transcript abundance of 3 out of 4 ALP subtypes relative to Wistar-Kyoto (WKY) rats; these are Germ line *(Alpg)*, Biomineralization associated *(Alpl)*, and Placental *(Alpp)*. Using RT-qPCR[28] to validate these

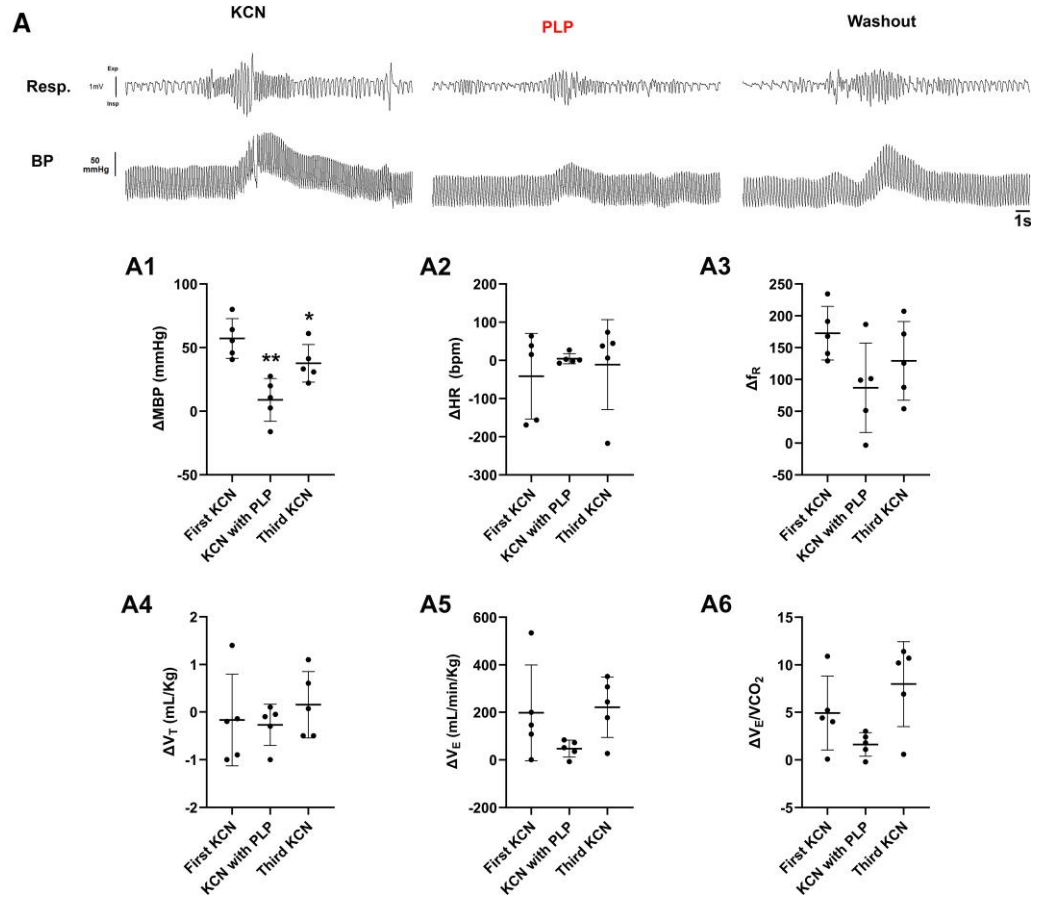


Figure 3 Effect of pyridoxal 5′ phosphate (PLP) on cardiorespiratory chemoreflexes. *A*) In adult telemetered SHR ($n = 5$ rats), we used potassium cyanide (KCN, 15 µg/rat; bolus injection i.v.) to stimulate the peripheral chemoreceptors either in the presence or absence of PLP (intravenous infusion—48 mg/kg/h, 30 min i.v.). At the top is a typical tracing of breathing in the whole-body plethysmography and blood pressure (BP) before, during, and after PLP infusion. PLP attenuated but did not abolish the KCN-evoked increase in mean blood pressure (MBP, A1). Other KCN-evoked responses quantified are bradycardia (HR, A2), tachypnoea (f_R, A3), changes in tidal volume (V_T, A4), minute ventilation (V_E, A5), and respiratory efficiency (V_E/VCO_2, A6). Data are shown as Δ response relative to the immediate baseline. Data were analysed using mixed regression model with either normal or gamma distribution. Mean ± SD, * P < 0.05, **P < 0.01.

results, we observed a 2-fold increase in mRNA expression for *Alpl* [$t_{(14)} = 14.0$, $P < 0.001$, Cohen'D = 6.98; *Figure* 4_{B2}] in the CB of SHR relative to Wistar rats, whereas both *Alpg* [$t_{(14)} = -2.64$, $P = 0.019$, Cohen'D = −1.32; *Figure* 4_{B1}] and pan-ALP—a primer pair recognizing multiple ALP isoforms (*Alpg*, *Alpp*, *Alpi*)—[$t_{(14)} = -3.17$, $P = 0.007$, Cohen'D = −1.59; *Figure* 4_{B3}] were slightly downregulated.

3.8 PHC attenuates the hypoxic ventilatory response (HVR) of human hypertensive patients with sensitised peripheral chemoreflex index

In a double-blind randomised placebo-controlled crossover study, 20 participants with hypertension stage 2 or above (i.e. averaged SBP = 151 ± 22 mmHg, DBP = 83 ± 8 mmHg) who met the inclusion criteria were recruited and attended three visits to our laboratory (for more information see Supplementary material online, *Section III-Additional Results*). The sample size was calculated *a priori* based on the work of Bock et al.[29] Participant demographics and current medications are reported in *Table 1*; baseline haemodynamic and blood biochemistry[30] are reported in Supplementary material online, *Table S3*. Using the isocapnic hypoxic rebreathing method,[31] an index of peripheral chemoreflex sensitivity was quantified via the change in patient's $\dot{V}_E$ relative to their estimated arterial oxygen saturation[32] (S_aO_2) (i.e. $\Delta\dot{V}_E/\Delta S_aO_2$). 4 participants did not complete the isocapnic hypoxic rebreathing due to technical issues, resulting to early termination; 1 participant had frequent ectopy and so the HVR test was not undertaken, and one participant withdrew after completing the PHC condition but not the placebo condition. Of the 14 participants completing the isocapnic hypoxic rebreathing test, half ($n = 7$) were deemed to have a 'sensitised' peripheral chemoreflex index (i.e. the response was greater than −0.5 L/min/% during the placebo visit). This stratification was based on the work of Narkiewicz et al.[7] Oral supplementation with 600 mg PHC, which is a dose sold over the counter in supplement formulations, significantly attenuated the HVR in patients with a sensitised peripheral chemoreflex index ($\Delta\dot{V}_E/\Delta S_aO_2$ $_{\text{treatment*level of chemosensitivity}} = 0.496$ L/min/% (95% CI, 0.09 to 0.90); $P = 0.021$, Supplementary material online, *Table S50*, *Figure 5*). In contrast, PHC had no effect on either resting blood pressure[33] or the hypoxic evoked pressor response ($P > 0.05$; Supplementary material online, *Tables S51* and *S52*, *Table 2*). Blood analysis of B6 vitamers indicates therapeutic concentrations in the micromolar range for pyridoxine, pyridoxal, and pyridoxic acid (see Supplementary

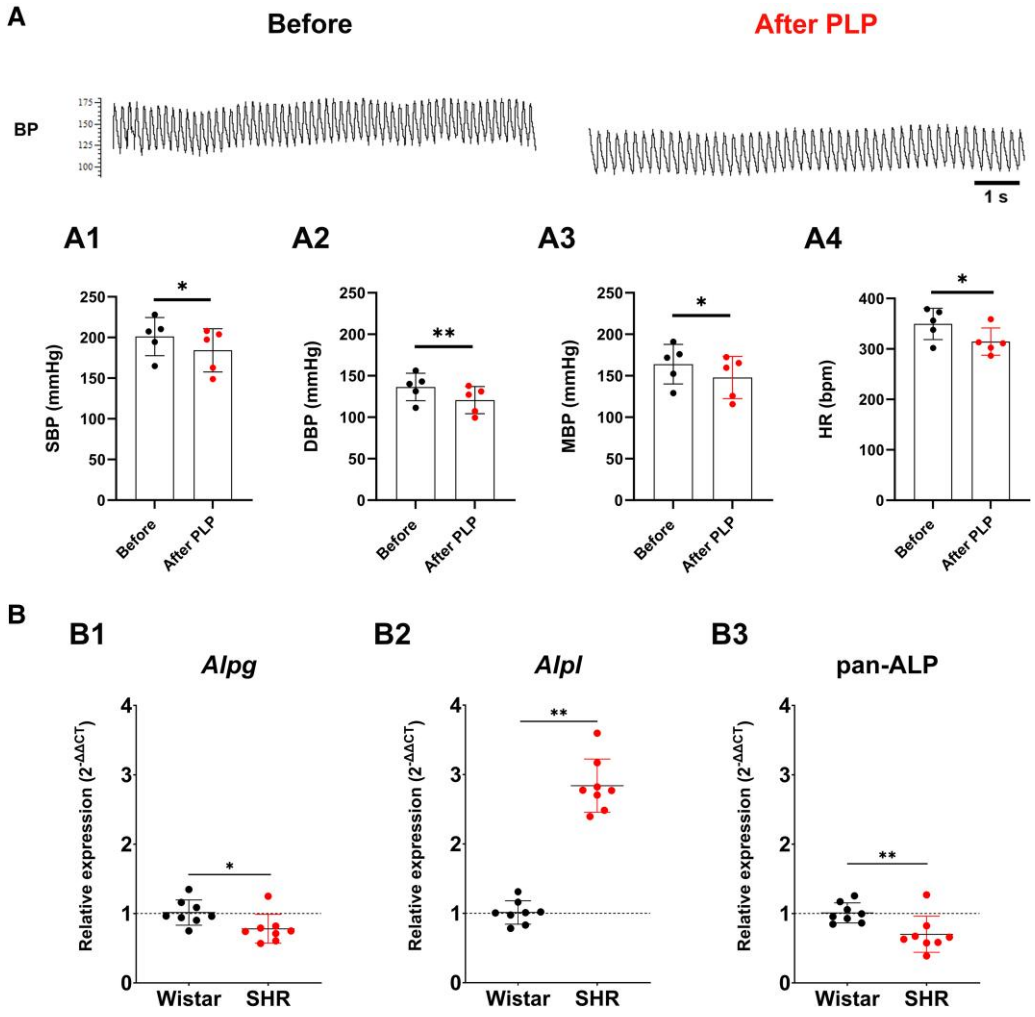


Figure 4 Effect of pyridoxal 5′ phosphate (PLP) on resting blood pressure *in vivo*. *A*) In adult SHRs ($n = 5$ rats), PLP was intravenously infused (48 mg/kg/h, 30 min) while recording the animal's BP. At the top is a typical tracing of BP before and immediately after the infusion. At the bottom, the effect of PLP infusion on resting SBP (A1), DBP (A2), MBP (A3) and HR (A4) *B*) RT-qPCR validation of *Alpl*, *Alpg*, and pan-ALP expression in the CB of male Wistar (4–6 weeks, $n = 8$ rats) and prehypertensive male (4–6 weeks, $n = 8$ rats) SHR. *Alpg* (B1), *Alpl* (B2), and pan-ALP (B3) expression were normalized to *Eif4b*—a housekeeping gene—and presented as relative change to Wistar. Data were analysed either independent *t*-test or mixed regression model with either normal or gamma distribution. Mean ± SD, * $P < 0.05$, ** $P < 0.01$.

material online, *Table S4*) about 2 h after the treatment, which allows us to infer that PLP also reached therapeutic concentrations.

4. Discussion

Our findings provide the first evidence that PLP, the active form of vitamin B6, binds to and antagonises the P2X3R of both SHR and humans with hypertension, thus attenuating CB hyperexcitability. Our *in vitro* data showed that PLP allosterically blocked $[Ca^{2+}]_i$ responses evoked by α, β-methylene-ATP in cell lines expressing recombinant hP2X3R. Its antagonist effect is supported by our *in silico* data, which predicts that PLP binds less strongly to the same negative allosteric pocket as MK-7264 (the highly potent antagonist Gefapixant). *In situ*, PLP attenuates both CB hypertonicity and hyperreflexia in SHR and attenuated preferentially the chemoreceptor sympathetic reflex. Likewise, *in vivo*, PLP preferentially attenuated the peripheral chemoreflex pressure response and lowered resting blood pressure in conscious SHR. In a small double-blind randomized clinical trial, oral supplementation with PHC, vitamin B6 attenuated peripheral chemoreflex respiratory sensitivity in participants with a sensitised chemoreflex index based on their HVR. This is the first study to demonstrate that blocking P2X3R attenuates the peripheral chemoreflex in humans.

In the field of drug discovery, computer-aided drug design (CADD) is an important method to identify molecular scaffolds with biological activity. Molecular docking is an *in silico* method to predict the three-dimensional orientation/conformation (i.e. binding pose) of a molecule within the binding site of a target of interest.[34] This technique, alongside molecular dynamic simulations, another *in silico* method for CADD, was recently used to investigate the interaction of MK-7264 and its analogues with the negative allosteric site of hP2X3R.[22] In this study, the authors reported that direct contacts of MK-7264 with Asn190 and Leu191 in hP2X3R play an important role in compound binding to the pocket. In addition, Gly189 seems to be critical for allosteric inhibition, even though MK-7264 did not display direct contacts with it in the 300 ns simulations we ran. Our molecular dynamic simulations predict a network of polar interactions with the residues defining the binding site, which include hydrogen bonds between oxygen atoms from MK-7264's sulphonamide group and Lys176. Comparatively, according to our molecular

Table 1 Hypertensive participant characteristics and medications

Demographics	
n	18 participants
Age (yr)	70 ± 8
Female, *n* (%)	12 (67)
Height (cm)	168 ± 10
Weight (kg)	76 ± 20
BMI (kg.m^{-2})	27 ± 5
Medication used	
ACE inhibitor, *n* (%)	5 (28)
Aldosterone receptor agonist, *n* (%)	1 (6)
Alpha blocker, *n* (%)	1 (6)
Anti-platelet, *n* (%)	2 (11)
Antipsychotic, *n* (%)	1 (6)
ARBs, *n* (%)	5 (28)
β blocker, *n* (%)	4 (22)
Ca^{2+} channel blocker, *n* (%)	8 (33)
Cholesterol lowering, *n* (%)	1 (6)
Islet enhancer/biguanide, *n* (%)	1 (6)
Statin, *n* (%)	7 (39)
Thiazide diuretic, *n* (%)	1 (6)
Thiazide-type diuretic, *n* (%)	4 (22)
Xanthine oxidase inhibitor, *n* (%)	1 (6)

Values are expressed as mean ± SD for continuous variables and frequency (%) for discrete variables. BMI: body mass index, ACE: angiotensin converting enzyme, ARB: angiotensin receptor blocker

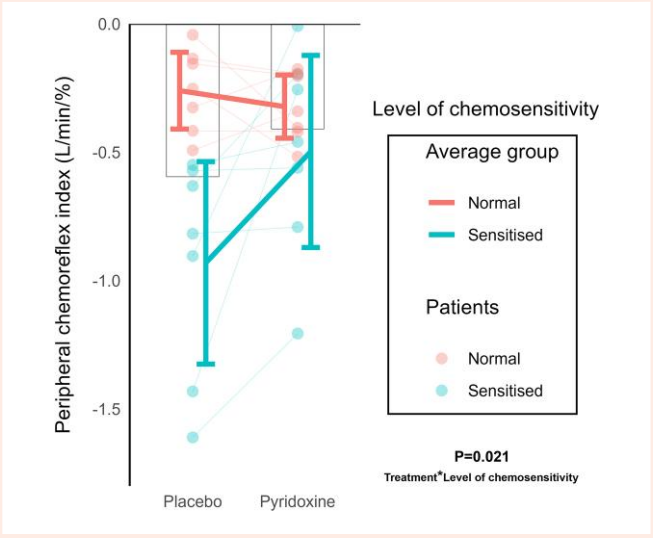


Figure 5 Effect of pyridoxine hydrochloride (PHC) on the index of peripheral chemoreflex sensitivity in patients with hypertension ($n = 14$ participants). Using the isocapnic hypoxic rebreathing method,[31] the index of peripheral chemoreflex sensitivity was quantified as the patients' peak $\dot{V}E$ response relative to their nadir arterial oxygen saturation (S_aO_2) (i.e. $\Delta\dot{V}E/\Delta SaO_2$). Half of the patients ($n = 7$ participants) were deemed to have a 'sensitized' index (i.e. the slope was steeper than −0.5 L/min/% during the placebo visit—this threshold was based on the work of Narkiewicz et al.[7]) Our analysis shows a significant interaction ($P = 0.021$) between the 'treatment' and 'level of chemosensitivity'. Data were analysed using a mixed regression model, with normal distribution. Mean ± 95% CI (confidence interval).

Table 2 Peripheral and central blood pressures with PHC supplementation in hypertensive participants

	Placebo	PHC	*P*-value
SBP (mmHg)	151 ± 22	157 ± 20	0.279
DBP (mmHg)	83 ± 8	86 ± 9	0.203
Mean pressure (mmHg)	115 ± 15	118 ± 12	0.456
Pulse pressure (mmHg)	69 ± 19	71 ± 18	0.437
Pulse rate (beats/min)	61 ± 8	62 ± 12	0.608
Central SBP (mmHg)	145 ± 20	148 ± 18	0.439
Central DBP (mmHg)	83 ± 9	86 ± 8	0.142
Central mean pressure (mmHg)	111 ± 12	114 ± 12	0.324
Central pulse pressure (mmHg)	62 ± 18	62 ± 15	0.981

Values are expressed as mean ± SD for $n = 14$ participants. SBP: systolic blood pressure, DBP: diastolic blood pressure. The main effect of treatment was assessed using a paired Student's *t*-test.

docking analyses, PLP is predicted to bind the upper portion of the pocket defined by MK-7264 with its phosphate group overlapping the sulphonamide function of MK-7264 (*Figure 1I*) and its pyridine ring occupying the space defined by the phenyl ring of MK-7264 (*Figure 1H, I*). Our molecular dynamics and binding energy calculations suggest PLP binds to the same binding pocket of MK-7264 albeit less strongly, supporting the relatively low potency of PLP in comparison to MK-7264 ($IC_{50} = 8.7$ μM vs. 42.6 nM,[35] respectively).

In the WHBP, intra-carotid bolus injections of PLP (1–5 mM) preferentially attenuated the CB-evoked sympathetic reflex over the respiratory and bradycardia responses. Similar results were observed with AF-353 in the same *in situ* preparation.[11] In addition, PLP lowered blood pressure in the SHR *in vivo*. This preferential response on the chemoreflex-evoked sympathoexcitation is not unique and was observed after denervating the sympathetic efferent to the CB[27] and following CB exposure to exedin-4,[20] a glucagon-like peptide-1 analogue. This sympathetic selectivity is consistent with the ribbon cable hypothesis that proposes separate lines of afferent transmission with different transmitters/receptors regulating distinct chemoreflex circuits governing separate target organs.[36] Similarly, intravenous infusions of PLP in telemetered conscious SHR preferentially attenuated the cardiovascular component of the peripheral chemoreflex response evoked by KCN (*Figure 3A*). It is important to acknowledge that we can observe attenuation trends in the respiratory component of the chemoreflex, e.g. the KCN-evoked tachypnoea was marginally attenuated ($P = 0.07$). In addition, in the present study, we quantified the neural inspiratory drive differently (i.e. PN amp/Ti), *in situ*, during KCN-evoked responses (see Supplementary material online, *Figure S6D*), which was not measured in the above-mentioned previous studies[11,20,27] but may be a more sensitive measure and explain the difference.

The effect of PLP on blood pressure of SHR was pronounced. While its ability to reduce blood pressure in hypertension is well-known,[18,37–39] its underlying mechanism is not. The fact that PLP could reduce blood pressure within 30 min of infusion supports its mechanism via P2X3R antagonism, as previously demonstrated with MK-7264.[11] However, this might not be the only mechanism by which PLP can reduce blood pressure if given chronically. Lellig et al.[40] investigated the ability of PLP to convert angiotensin II (Ang II) into pyruvamide–Ang II, which has less affinity to Ang II type 1 receptors (AT1R). Using an osmotic pump, the authors delivered PLP at a dose of 4 μg/Kg/day in SHR; after 3 days of treatment, the authors observed significant falls in both SBP and DBP. In addition, the authors treated WKY rats with Ang II to induce hypertension, which was blocked in PLP-treated rats. These results indicate that PLP could lead to falls in blood pressure via synergic mechanisms (i.e. P2X3R antagonism and scavenging of Ang II) depending on the dose. Despite that, the authors did not demonstrate increased levels of

pyruvamide–Ang II in either the blood or urine of PLP-treated rats, therefore, not confirming their theory that such a reaction happens, at significant levels, *in vivo*.

PLP infusion produced a small reduction in VCO_2 without changes in breathing (see Supplementary material online, *Table S2*). Since VO_2 was unchanged, this led to a reduction in the respiratory exchange ratio (RER) favouring lipid oxidation. This is possible since PLP is a co-factor in many metabolic reactions, including carbohydrate and lipid catabolism.[41–44] For instance, Zemel & Bruckbauer[43] demonstrated that PHC increased fat oxidation and reduced RER in overweight/obese patients.

In our experiments, the effect of PLP on CB activity was relatively short-lived with recovery around 30 min after the end of the infusion. The latter reflects the known PLP pharmacokinetics with a half-life ($T_{1/2}$) of 2.3 h in humans,[45] 49 min in goats, and 16 min in pigs.[46] PLP is metabolized by two enzymes, PLP Phosphatase and ALP.[47] Interestingly, increased serum ALP activity is associated with both low levels of PLP[48] and increased risk for cardiovascular disease,[49] which includes hypertension.[50] Therefore, adequate ALP activity seems pivotal for maintaining physiological blood levels of PLP. Herein, we report higher levels of mRNA expression for *Alpl* in the CB of SHR. Whether the increased *Alpl* gene expression is translated into increased enzymatic activity that reduces PLP translation in the CB of SHR is unknown but might explain limitations on $T_{1/2}$.

Translating these studies, we carried out a small double-blind randomised clinical trial to investigate the capability of PLP to attenuate CB hyperexcitability in patients with hypertension. Using the index of peripheral chemosensitivity from the placebo visit, we categorised patients having either sensitised or normal CB activity. This categorisation was based on collective work from the literature, which defines a normal response as approximately −0.35 L/min/%—for review see Felippe et al.[3] In addition, Narkiewicz et al.[7] were able to sort patients with hypertension who underwent unilateral CB resection into responders and non-responders based on their baseline HVR (-0.50 ± 0.05 and -0.32 ± 0.06 L/min/%, respectively). Interestingly, PHC's ability to attenuate the HVR occurred preferentially in patients with sensitised peripheral chemoreflex index (i.e. steeper than −0.5 L/min/%). In contrast to our rodent SHR studies, we did not see any effect on either resting blood pressure or the hypoxic pressor response. The reason for that might be that the hypertension was mild or reflected the acute, single dose, regimen of our protocol; however, when given chronically, oral supplementation with PHC reduced blood pressure and circulating catecholamines in hypertensive patients.[18,39] It is worth mentioning that this is the first study to show that blocking P2X3R can attenuate the peripheral chemoreflex sensitivity in humans, thus reproducing previous animal work with MK-7264.[11,12]

Our initial study protocol, as per trial registration in 2020, involved a longitudinal study with different endpoints and plans to record the peroneal muscle sympathetic nerve activity using microneurography; however, the study protocol was negatively impacted by the COVID-19 pandemics. Thus, after amending the study with the ethics committee, we changed our protocol to the one presented herein. Therefore, not being able to carry out the protocol as initially registered is a clear limitation of our study. As another limitation, we were not successful to recruit a diverse cohort of participants; this compromised our ability to investigate whether there are any sex and race/ethnicity-specific responses to PHC supplementation.

It is important to recognise inter-species differences between humans and rodents in the responses to vitamin B6 treatment. Even though P2X3R is highly homologous between rat and humans (i.e. 97%),[51] there are differences between the receptor conductance and kinetics of these two species.[51,52] Additionally, when treated with vitamin B6, rats (SHR) showed an acute fall in blood pressure; humans did not, and rats seemed to need a higher concentration of plasma PLP and to have a shorter $T_{1/2}$. Finally, the respiratory component of the chemoreflex in rats seems to be less sensitive to vitamin B6 treatment than humans.

In addition to these inter-species differences, we acknowledge that the aetiology of hypertension in humans is not necessarily identical to that of the SHR. Specifically, it remains unknown to what extent the increase in sympathetic outflow mediated by CB hyperexcitability contributes to the high blood pressure phenotype in human patients. In support, we note that an increase in sympathetic activity has been reported in approximately 60% of patients with essential hypertension[53] and previous clinical trials of CB resection prove that the CB is a viable target to treat autonomic imbalance in patients with resistant hypertension.[7] The latter study reproduced findings in SHR[54], which demonstrates that SHRs is a good animal model for translating CB interventions.

We speculate that one reason that the B6 treatment did not lower blood pressure in our human study may relate to the relatively mild hypertension in these patients. Although patients were asked to abstain from any cardioactive medications on the morning of the study, it is unlikely that their medications were completely cleared from the bloodstream, which could act as confound variable. Finally, in humans, we used oral PHC instead of intravenous PLP as used in the rats, which means that PHC needed to be absorbed and converted into PLP and reach blood concentration at micromolar levels. Although blood analysis of B6 indicated therapeutic concentrations in the micromolar range for pyridoxamine, pyridoxal, and pyridoxic acid in our human subjects, the blood concentration of PLP in SHR was probably higher, since animals received direct intravenous infusions of PLP (24 mg/Kg). This higher concentration might be the reason why the blood pressure falls acutely in SHRs rather than in humans. Nonetheless, as previously stated, it is important to note that clinical trials with B6 supplementation in patients with hypertension showed that the latter can decrease blood pressure in humans when given chronically.[18,39]

Altogether, our experiments support that PLP can attenuate CB hyperexcitability in hypertension of both rats and humans. Most importantly, the attenuating effect does not fully block the chemoreflex and showed selectivity for patients with a sensitised peripheral chemoreflex index. Given that an effective dose is sold over the counter as a supplement relatively cheaply, this could have meaningful therapeutic implications especially in developing economies (see Lellig et al.[40] for further discussion on the topic). It is important to acknowledge possible adverse side effects of long-term supplementation with vitamin B6, such as peripheral neuropathy, which could limit its therapeutic use and should be taken into consideration by healthcare providers.[55,56] Nevertheless, our findings suggest that PLP is a viable candidate for larger clinical trials to treat CB dysregulation in cardiovascular disease, such as hypertension, heart failure, sleep apnoea, and possibly non-CB related disease, for instance, refractory chronic cough.

Translational perspective

What is new?

- This is the first study to demonstrate that blocking P2X3R in humans attenuates their peripheral chemoreflex sensitivity.
- We show for the first time that PLP antagonizes P2X3R in the CB.
- PLP attenuates high peripheral chemoreflex sensitivity in patients with hypertension at therapeutic doses.

What are the clinical implications?

- The findings indicate that supplementation of vitamin B6 is capable of selectively attenuating CB dysregulation in people with hypertension who have a sensitised peripheral chemoreflex index.

Supplementary material

Supplementary material is available at *Cardiovascular Research* online.

Authors' contributions

Conceptualization: JFRP, JPF, MD. Methodology: ISAF, SJF, MB, AP, FM, JPF. Investigation (Data Collection): ISAF, TLB, RS, MB, JLF, AP, OG, PT, MD. Data analysis and interpretation: ISAF, TLB, RS, MB, MLB, AP, OG. Project administration: JFRP, JPF, ISAF, TLB. Supervision: JFRP, JPF, SJF. Writing—original draft: ISAF. Writing—review & editing: ISAF, JFRP, JPF, MLB, MB, SJF, AP, JLF, TLB.

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Data availability

The authors declare that all supporting data have been made publicly available at Figshare and can be accessed using DOI (http://dx.doi.org/10.17608/k6.auckland.28191725). Raw data is available from the corresponding author upon reasonable request.

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