

Involvement of β -adrenoceptors in the cardiovascular responses induced by selective adenosine A_{2A} and A_{2B} receptor agonists

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Abstract

A_{2A} and A_{2B} adenosine receptors produce regionally selective regulation of vascular tone and elicit differing effects on mean arterial pressure (MAP), whilst inducing tachycardia. The tachycardia induced by the stimulation of A_{2A} or A_{2B} receptors has been suggested to be mediated by a reflex increase in sympathetic activity. Here, we have investigated the role of β_1 - and β_2 -adrenoceptors in mediating the different cardiovascular responses to selective A_{2A} and A_{2B} receptor stimulation. Hemodynamic variables were measured in conscious male Sprague-Dawley rats (350–450 g) via pulsed Doppler flowmetry. The effect of intravenous infusion (3 min per dose) of the A_{2A} -selective agonist CGS 21680 (0.1, 0.3, 1.0 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) or the A_{2B} -selective agonist BAY 60–6583 (4.0, 13.3, 40.0 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) in the absence or following pre-treatment with the non-selective β -antagonist propranolol (1.0 $\text{mg}\cdot\text{kg}^{-1}$), the selective β_1 -antagonist CGP 20712A (200 $\mu\text{g}\cdot\text{kg}^{-1}$), or the selective β_2 -antagonist ICI 118,551 (2.0 $\text{mg}\cdot\text{kg}^{-1}$) was investigated (maintenance doses also administered). CGP 20712A and propranolol significantly reduced the tachycardic response to CGS 21680, with no change in the effect on MAP. ICI 118,551 increased BAY 60–6583-mediated renal and mesenteric flows, but did not affect the heart rate response. CGP 20712A attenuated the BAY 60–6583-induced tachycardia. These data imply a direct stimulation of the sympathetic activity via cardiac β_1 -adrenoceptors as a mechanism for the A_{2A} - and A_{2B} -induced tachycardia. However, the regionally selective effects of A_{2B} agonists on vascular conductance were independent of sympathetic activity and may be exploitable for the treatment of acute kidney injury and mesenteric ischemia.

KEYWORDS

A_{2A} receptor, A_{2B} receptor, adenosine, hemodynamics, β -adrenoceptor

Abbreviations: A_1 R, Adenosine A_1 receptor; A_{2A} R, Adenosine A_{2A} receptor; A_{2B} R, Adenosine A_{2B} receptor; A_3 R, adenosine A_3 receptor; BRET, bioluminescence resonance energy transfer; cAMP, cyclic adenosine monophosphate; GPCR, G protein-coupled receptor; HR, heart rate; HVC, hindquarters vascular conductance; MAP, mean arterial pressure; MVC, mesenteric vascular conductance; NTS, nucleus of the solitary tract; RVC, renal vascular conductance; VC, vascular conductance.

Edward S. Wragg and Patrizia Pannucci contributed equally to this work

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1 | INTRODUCTION

Adenosine is a purine nucleoside that has an important role within the cardiovascular system.^{1,2} The physiological actions of adenosine are the consequence of its interaction with four different G protein-coupled receptors (GPCRs), namely the adenosine A_{1} , A_{2A} , A_{2B} , and A_{3} receptors ($A_{1}R$, A_{2AR} , A_{2BR} , A_{3R}).^{3,4} By interacting with these GPCRs, adenosine and its analogues initiate defined signaling pathways that provoke different biological effects on numerous organ systems.^{5,6} A_{1} and A_{3} adenosine receptors primarily couple to the inhibitory G-proteins G_o or G_i suppressing cyclic adenosine monophosphate (cAMP) production, while, in contrast, A_{2A} and A_{2B} subtypes preferentially activate stimulatory G_s proteins, thus increasing intracellular cAMP concentrations.^{4,7} A_{2A} and A_{2B} receptors are widely expressed in the cardiovascular system, where their regulation plays a key modulatory role in controlling heart rate and blood pressure.⁸⁻¹⁰ In addition to regulating heart rate (HR), cardiac contraction, inflammation, and vascular remodeling,^{3,11} both A_{2A} and A_{2B} receptors mediate systemic and pulmonary vasodilation.^{12,13} Activation of these adenosine receptors in response to hypoxic or ischemic stress also plays an important role in the prevention of renal failure by promoting renal perfusion.^{7,14} A_{2A} and A_{2B} receptors are therefore promising targets for a wide range of cardiovascular diseases, in particular hypertension and acute kidney injury.^{7,14,15}

An in vivo evaluation of the cardiovascular effects of selective A_{2A} and A_{2B} agonists, CGS 21680 and BAY 60-6583, respectively, showed that A_{2A} and A_{2B} receptors exert a regionally selective control of vascular conductance.¹⁰ The $A_{2A}R$ subtype mediates vasodilatory effects in the hindquarters vascular bed, with minimal impact on the mesenteric and renal vasculature, whereas A_{2BR} s have been demonstrated to have major control of renal and mesenteric vascular tone but have no effect on hindquarters vascular conductance.¹⁰ In addition, the activation of both A_2 receptor subtypes resulted in a parallel increase in HR, which may be secondary to a reflex response to the vasodilatation induced in different vascular beds.¹⁰

The arterial baroreflex is a neural mechanism that plays a crucial role in the fine regulation of blood pressure.¹⁶ The continuous sensation of blood pressure by tonic arterial baroreceptors allows this reflex to make constant adjustments of blood pressure by inducing rapid changes in heart rate and peripheral vascular resistance.¹⁷ Within the context of the neural control of cardiovascular function, the nucleus of the solitary tract (NTS) represents the first synaptic station for the processing of cardiovascular afferent inputs.¹⁸ In this regard, there is evidence of A_{2A} receptor involvement in the control of baroreflex activity via NTS,¹⁹ as well as A_{2B} receptor involvement in cardiovascular regulation via the posterior hypothalamus.²⁰

Sympathetic activity and its effect on the cardiovascular system are crucial aspects of the baroreceptor reflex.¹⁶ Indeed, the modulation of the sympathetic outflow is under the control of baroreceptor afferent activity, which evokes changes in the sympathetic activity to maintain an adequate blood pressure.²¹ The physiological responses to sympathetic activation result from the interaction between catecholamines and adrenoceptors.²² In particular,

β_1 and β_2 adrenoceptors play fundamental roles in the regulation of cardiovascular homeostasis.^{23,24} To evaluate the contribution of β adrenoceptors to the tachycardia induced by selective A_{2A} and A_{2B} receptor stimulation, we have used the non-selective β antagonist (propranolol), a β_1 antagonist (CGP 20712A) and a β_2 antagonist (ICI 118,551) to evaluate the extent to which the chronotropic and vasodilatory effects induced by selective A_{2A} (CGS 21680) and A_{2B} (BAY 60-6583) agonists result from an increase in sympathetic activity.

2 | MATERIALS AND METHODS

2.1 | Drugs, chemical reagents, and other material

4-[2-[[6-Amino-9-(N-ethyl- β -D-ribofuranuronamidoyl)-9H-purin-2-yl]amino]ethyl]benzenepropanoic acid hydrochloride (CGS 21680 hydrochloride) (Cat#1063), 2-[[6-Amino-3,5-dicyano-4-[4-(cyclopropylmethoxy)phenyl]-2-pyridinyl]thio]-acetamide (BAY 60-6583) (Cat#4472), (R)-1-[[1-Methylethyl]amino]-3-(1-naphthalenyloxy)-2-propanol hydrochloride (propranolol hydrochloride) (Cat#0624), 1-[2-[(3-Carbamoyl-4-hydroxy)phenoxy]ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol dihydrochloride (CGP 20712A dihydrochloride) (Cat#1024) and (\pm)-erythro-(S*,S*)-1-[2,3-(Dihydro-7-methyl-1H-inden-4-yl)oxy]-3-[[1-methylethyl]amino]-2-butanol hydrochloride (ICI 118,551 hydrochloride) (Cat#0821) were purchased from Tocris Bioscience (Bristol, UK). The fluorescent ligand, CA200645, was purchased from Hello Bio, (Bristol, UK) (Cat#HB7812). Dimethyl Sulfoxide (DMSO) (Cat#D5879) and Bovine Serum Albumin (BSA) (Cat#A7030) were purchased from Sigma-Aldrich (Gillingham, UK). Furimazine (Cat#N1130) was purchased from Promega (Madison, WI, USA).

Fentanyl citrate was acquired from Martindale Pharmaceuticals (Essex, UK). Buprenorphine (Buprecare), Medetomidine Hydrochloride (Sedastart) and Atipamezole Hydrochloride (Sedastop) were obtained from Animalcare Ltd. (York, UK). Meloxicam (Metacam) was acquired from Boehringer Ingelheim Animal Health (Berkshire, UK). Heparin Sodium was obtained from Wockhardt (Wrexham, UK). Pentobarbitone (Euthatal) was obtained from Alstoe Animal Health (York, UK). Tween 80 (Cat#P1754) and propylene glycol (Cat#P4347) were purchased from Sigma-Aldrich (Gillingham, UK).

2.2 | Animals and surgery

Male Sprague-Dawley rats (Charles River Laboratories, UK; 350–450 g) were used to perform these experiments. Animals were housed in pairs in a temperature-controlled room (21–23°C) with a 12 h light-dark cycle (lights on at 06:00) with free access to food (18% Protein Rodent Diet; Envigo, Madison WI, USA) and water. Upon arrival within the Unit, animals were housed during an acclimatization period of at least of 7 days prior to any surgery. All procedures were performed with approval from the University of Nottingham Animal Welfare and Ethical Review Board and performed in line with the Animals (Scientific Procedures) Act (1986), under UK Home Office approved Project License and Personal License

authority. 53 rats were used during this study, and all animal experiments are reported in compliance with the ARRIVE guidelines²⁵ and the editorial on reporting animal studies.²⁶ Surgical procedures were carried out under general anesthesia (fentanyl and medetomidine, 300 $\mu\text{g}\cdot\text{kg}^{-1}$ each, i.p., supplemented as required). During the first surgery, miniature pulsed Doppler flow probes were implanted around the left renal and superior mesenteric arteries and the descending abdominal aorta to monitor haemodynamics.²⁷ The probe wires were led subcutaneously to the nape of the neck, where they were taped and secured. Atipamezole hydrochloride (1 $\text{mg}\cdot\text{kg}^{-1}$, s.c.) and buprenorphine (30 $\mu\text{g}\cdot\text{kg}^{-1}$, s.c.) were provided as reversal agents and postoperative analgesia. A second dose of analgesia (buprenorphine 15 $\mu\text{g}\cdot\text{kg}^{-1}$, s.c.) was given 4 h post-surgery. Supplementary analgesia (Meloxicam, 1 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, s.c.) was administered for 3 days after surgery.

A second surgery was carried out at least 10 days after the surgical implantation of the vascular probes and after a satisfactory welfare inspection from the Named Veterinary Surgeon. During this surgery, performed under anesthesia (fentanyl and medetomidine, 300 $\mu\text{g}\cdot\text{kg}^{-1}$ each, i.p., supplemented as required), a catheter was implanted into the distal abdominal aorta via the caudal artery (to measure arterial blood pressure and heart rate), and three catheters were implanted into the right jugular vein (for drug administration).²⁷ All catheters were led subcutaneously to the nape of the neck.

The probe wires were released from the nape of the neck to be soldered into a miniature plug (Omnetic connector corporation, USA), which was then mounted onto a custom-designed harness worn by the rat. The catheters and probe wires were protected by a spring secured to the harness and attached to a counterbalanced pivot system to allow the free movement of the animal. Reversal of anesthetic and analgesia was administered (as described above). The arterial catheter was filled and infused with heparinized (15 $\text{U}\cdot\text{ml}^{-1}$) saline overnight to maintain potency.

Experiments began 24 h after surgery for catheter implantation, with animals fully conscious and unrestrained in home cages, with free access to food and water.

2.3 | Cardiovascular recordings

During the cardiovascular monitoring periods, rats were connected to the customized data-acquisition software (see below) via a tether system. Recordings were made for at least 30 min prior to the administration of any interventions and continuously for a minimum of 4 h thereafter. HR, mean arterial blood pressure (MAP), renal, mesenteric, and hindquarters Doppler shifts were measured by a transducer amplifier (13-4615-50; Gould, Cleveland, OH, USA), a Doppler flowmeter (Crystal Biotech, Holliston, MA, USA), and a VF-1 mainframe (pulse repetition frequency 125 kHz) fitted with high-velocity (HVPD-20) modules. These measurements were recorded by customized computer software (IdeeQ; Maastricht Instruments, Maastricht, The Netherlands). Raw data were sampled by IdeeQ every 2 ms, averaged, and stored to disc every cardiac cycle. Changes in vascular conductance in the renal (RVC), mesenteric (MVC), and hindquarter

(HVC) vascular beds, respectively, were calculated from the changes in MAP and Doppler shift.

2.4 | Experimental protocol

Experiments were run in six studies, each lasting 3 days; within each study was a contemporaneous vehicle control (5% propylene glycol, 2% Tween 80 in sterile saline). Experiments were run with treatment groups of 8 to 10 rats.

2.4.1 | Study 1: The effect of β_1 antagonist CGP 20712A (200 $\mu\text{g}\cdot\text{kg}^{-1}$ bolus; 100 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, 90 min infusion) on the hemodynamic profile of A_{2A} agonist CGS 21680

Eight animals were used to measure the cardiovascular responses to CGS 21680 in the presence or absence of CGP 20712A. Following a period of baseline recordings, rats were randomized into two groups. **Group 1** received vehicle intravenous bolus (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (0.4 $\text{ml}\cdot\text{h}^{-1}$) on day 1 and CGP 20712A intravenous bolus (200 $\mu\text{g}\cdot\text{kg}^{-1}$) (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (100 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) (0.4 $\text{ml}\cdot\text{h}^{-1}$) on day 3. **Group 2** received CGP 20712A (200 $\mu\text{g}\cdot\text{kg}^{-1}$, 0.1 ml i.v. bolus; 100 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, 0.4 $\text{ml}\cdot\text{h}^{-1}$ i.v. infusion) on day 1 and vehicle (0.1 ml i.v. bolus; 0.4 $\text{ml}\cdot\text{h}^{-1}$ i.v. infusion) on day 3. After 90 min all groups received intravenous infusions (0.1 $\text{ml}\cdot\text{min}^{-1}$) of CGS 21680 (0.1 (low), 0.3 (mid), and 1.0 (high) $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Each dose of CGS 21680 was given as a 3 min infusion. Cardiovascular recordings were continued for a further 4 h after administration of CGS 21680.

2.4.2 | Study 2: The effect of β_2 antagonist ICI 118 551 (2.0 $\text{mg}\cdot\text{kg}^{-1}$ bolus; 1.0 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, 90 min infusion) on the hemodynamic profile of A_{2A} agonist CGS 21680

Nine animals were used to assess the cardiovascular responses to CGS 21680 in the presence or absence of ICI 118,551. After a period of baseline recordings, rats were randomized into two groups. **Group 1** received vehicle intravenous bolus (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (0.4 $\text{ml}\cdot\text{h}^{-1}$) on day 1 and an ICI 118,551 intravenous bolus (2.0 $\text{mg}\cdot\text{kg}^{-1}$) (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (1.0 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) (0.4 $\text{ml}\cdot\text{h}^{-1}$) on day 3. **Group 2** received ICI 118,551 (2.0 $\text{mg}\cdot\text{kg}^{-1}$, 0.1 ml i.v. bolus; 1 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, 0.4 $\text{ml}\cdot\text{h}^{-1}$ i.v. infusion) on day 1 and vehicle (0.1 ml i.v. bolus; 0.4 $\text{ml}\cdot\text{h}^{-1}$ i.v. infusion) on day 3. Approximately 90 min after the initial bolus of vehicle or ICI 118,551, all groups received intravenous infusions (0.1 $\text{ml}\cdot\text{min}^{-1}$) of CGS 21680 (0.1 (low), 0.3 (mid), and 1.0 (high) $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). Each dose was infused for 3 min. Hemodynamic recordings were made for a further 4 h following the completion of the CGS 21680 intravenous infusion period.

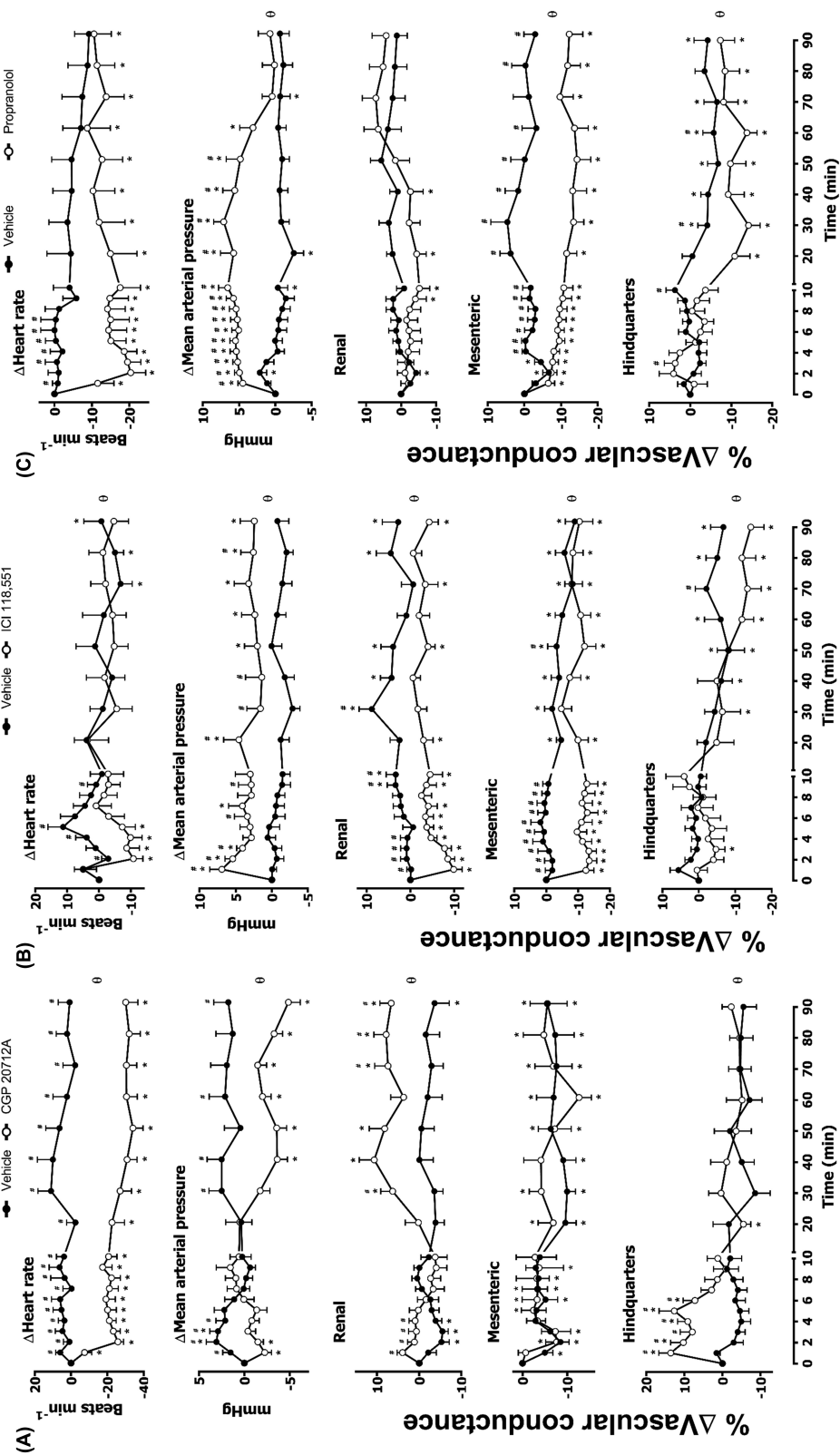


FIGURE 1 Cardiovascular responses to (A) CGP 20712A, (B) ICI 118,551, or (C) propranolol in conscious, freely moving rats. Rats were dosed with either (A) CGP 20712A (0.1 ml bolus dose of 200 $\mu\text{g}\cdot\text{kg}^{-1}$, followed by a 90 min infusion of 100 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, i.v., $n = 16$) or vehicle (0.1 ml bolus dose of 5% propylene glycol, 2% Tween 80 in sterile saline, $n = 16$); (B) ICI 118,551 (0.1 ml bolus dose of 2.0 $\text{mg}\cdot\text{kg}^{-1}$, followed by a 90 min infusion of 1.0 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, i.v., $n = 17$) or vehicle; or (C) propranolol (0.1 ml bolus dose of 1.0 $\text{mg}\cdot\text{kg}^{-1}$, followed by a 90 min infusion of 0.5 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$, i.v., $n = 20$) or vehicle, as described in the methods. The time course shows the responses over the 90 min period during the infusion. Data points are mean and vertical bars represent SEM. * $p < .05$ versus baseline (Friedman's test). A Wilcoxon signed-rank test was conducted between treated and vehicle control groups for a comparison of area under/over the curve ($0 p < .05$) and to determine differences at each time point ($\# p < .05$, Wilcoxon T-test equivalent).

TABLE 1 Cardiovascular variables prior to administration of β -antagonists for combined study datasets

Baseline (t = 0)	Combination of Studies 1 & 4			Combination of Studies 2 & 5			Combination of Studies 3 & 6			
	Vehicle			Vehicle			Vehicle			
	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n	Mean \pm SEM	n
HR (beats min ⁻¹)	333 \pm 5	16	338 \pm 8	16	330 \pm 6	17	332 \pm 7	17	348 \pm 5	20
MAP (mmHg)	102 \pm 2	16	104 \pm 2	16	104 \pm 3	17	103 \pm 2	17	104 \pm 1	20
RVC (U)	81 \pm 7	15	73 \pm 5	15	93 \pm 8	16	89 \pm 7	16	83 \pm 7	16
MVC (U)	82 \pm 7	14	75 \pm 8	14	87 \pm 7	17	83 \pm 5	17	93 \pm 7	18
HVC (U)	44 \pm 5	14	42 \pm 4	14	44 \pm 4	16	43 \pm 4	16	54 \pm 5	19

Note: Values are mean \pm SEM. Units of vascular conductance (VC) are kHz. $\text{mmHg}^{-1} \times 10^3$. n = 14–20 per combined group.

Wilcoxon matched-pairs signed-rank test. *p < .05 versus corresponding vehicle group.

Abbreviations: HR, heart rate; HVC, hindquarters vascular conductance; MAP, mean arterial pressure; MVC, mesenteric vascular conductance; RVC, renal vascular conductance; U, units; VC, vascular conductance.

2.4.3 | Study 3: The effect of β antagonist propranolol (1.0 mg.kg⁻¹ bolus; 0.5 mg.kg⁻¹.h⁻¹, 90 min infusion) on the hemodynamic profile of A_{2A} agonist CGS 21680

Ten animals were used to assess the cardiovascular responses to CGS 21680 in the presence or absence of propranolol. After a period of baseline recordings, rats were randomized into two groups. **Group 1** received vehicle intravenous bolus (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (0.4 ml.h⁻¹) on day 1 and a propranolol intravenous bolus (1.0 mg.kg⁻¹) (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (0.5 mg.kg⁻¹.h⁻¹) (0.4 ml.h⁻¹) on day 3. **Group 2** received propranolol (1.0 mg.kg⁻¹, 0.1 ml i.v. bolus; 0.5 mg.kg⁻¹.h⁻¹, 0.4 ml.h⁻¹ i.v. infusion) on day 1 and vehicle (0.1 ml i.v. bolus; 0.4 ml.h⁻¹ i.v. infusion) on day 3. Approximately 90 min after the initial bolus of vehicle or propranolol, all groups received intravenous infusions (0.1 ml.min⁻¹) of CGS 21680 (0.1 (low), 0.3 (mid), and 1.0 (high) $\mu\text{g.kg}^{-1} \text{min}^{-1}$). Each dose was infused for 3 min. Hemodynamic recordings were made for a further 4 h following the completion of the CGS 21680 intravenous infusion period.

2.4.4 | Study 4: The effect of β_1 antagonist CGP 20712A (200 $\mu\text{g.kg}^{-1}$ bolus; 100 $\mu\text{g.kg}^{-1}$.h⁻¹, 90 min infusion) on the hemodynamic profile of A_{2B} agonist BAY 60–6583

Eight animals were used to measure the cardiovascular responses to BAY 60–6583 in the presence or absence of CGP 20712A. Following a period of baseline, rats were randomized into two groups. **Group 1** received vehicle intravenous bolus (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (0.4 ml.h⁻¹) on day 1 and CGP 20712A intravenous bolus (200 $\mu\text{g.kg}^{-1}$) (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (100 $\mu\text{g.kg}^{-1}$.h⁻¹) (0.4 ml.h⁻¹) on day 3. **Group 2** received CGP 20712A (200 $\mu\text{g.kg}^{-1}$, 0.1 ml i.v. bolus; 100 $\mu\text{g.kg}^{-1}$.h⁻¹, 0.4 ml.h⁻¹ i.v. infusion) on day 1 and vehicle (0.1 ml i.v. bolus; 0.4 ml.h⁻¹ i.v. infusion) on day 3. After 90 min all groups received intravenous infusions (0.1 ml.min⁻¹) of BAY 60–6583 (4.0 (low), 13.3 (mid), and 40.0 (high) $\mu\text{g.kg}^{-1} \text{min}^{-1}$). Each dose of BAY 60–6583 was given as a 3 min infusion. Cardiovascular recordings were continued for a further 4 h after administration of BAY 60–6583.

2.4.5 | Study 5: The effect of β_2 antagonist ICI 118,551 (2.0 mg.kg⁻¹ bolus; 1.0 mg.kg⁻¹.h⁻¹, 90 min infusion) on the haemodynamic profile of A_{2B} agonist BAY 60–6583

Eight animals were used to assess the cardiovascular responses to BAY 60–6583 in the presence or absence of ICI 118,551. After a period of baseline recordings, rats were randomized into two groups. **Group 1** received vehicle intravenous bolus (0.1 ml provided over

TABLE 2 Cardiovascular variables prior to administration of β -antagonists (top). Cardiovascular variables prior to infusion of A_{2A} agonist CGS 21680 (Study 1, 2, 3) or A_{2B} agonist BAY 60–6583 (Study 4, 5, 6) (bottom)

Baseline (t = 0)	Study 1			Study 2			Study 3			Study 4			Study 5			Study 6												
	CGP 20712A			Vehicle			ICI 118,551			Propranolol			CGP 20712A			Vehicle			ICI 118,551			Vehicle			Propranolol			
	Mean \pm SEM	n	n	Mean \pm SEM	n	n	Mean \pm SEM	n	n	Mean \pm SEM	n	n	Mean \pm SEM	n	n	Mean \pm SEM	n	n	Mean \pm SEM	n	n	Mean \pm SEM	n	n	Mean \pm SEM	n	n	
HR (beats min ⁻¹)	337 \pm 8	8	328 \pm 11	8	320 \pm 5	8	320 \pm 8	9	350 \pm 7	10	344 \pm 7	10	329 \pm 7	10	348 \pm 12	8	341 \pm 12	8	346 \pm 9	8	346 \pm 6	10	343 \pm 7	10	343 \pm 7	10	343 \pm 7	10
MAP (mmHg)	101 \pm 2	8	107 \pm 3	8	101 \pm 3	8	97 \pm 2	9	105 \pm 3	10	104 \pm 3	10	102 \pm 3	10	102 \pm 1	8	109 \pm 5	8	110 \pm 3	8	103 \pm 1	10	102 \pm 3	10	102 \pm 3	10	102 \pm 3	10
RVC (U)	83 \pm 10	7	69 \pm 8*	7	88 \pm 9	7	92 \pm 8	9	85 \pm 7	10	86 \pm 7	10	79 \pm 9	10	78 \pm 6	8	100 \pm 16	7	86 \pm 12	7	83 \pm 12	6	84 \pm 7	6	84 \pm 7	6	84 \pm 7	6
MVC (U)	84 \pm 7	7	73 \pm 7	7	97 \pm 10	7	89 \pm 8	9	80 \pm 7	9	103 \pm 8	9	80 \pm 13	9	78 \pm 15	7	76 \pm 8	8	76 \pm 6	8	106 \pm 11	9	102 \pm 12	9	102 \pm 12	9	102 \pm 12	9
HVC (U)	36 \pm 7	7	32 \pm 5	7	48 \pm 3	7	48 \pm 4	9	51 \pm 5	10	53 \pm 7	10	53 \pm 6	10	52 \pm 5	7	39 \pm 7	7	37 \pm 7	7	55 \pm 8	9	55 \pm 8	9	55 \pm 8	9	55 \pm 8	9
HR (beats min ⁻¹)	329 \pm 9	8	303 \pm 9*	8	312 \pm 5	8	316 \pm 8	9	341 \pm 7	10	333 \pm 8	10	331 \pm 10	10	309 \pm 8	8	340 \pm 11	8	334 \pm 11	8	336 \pm 8	10	332 \pm 8	10	332 \pm 8	10	332 \pm 8	10
MAP (mmHg)	103 \pm 1	8	99 \pm 3	8	99 \pm 2	8	100 \pm 3	9	104 \pm 2	10	101 \pm 3	10	103 \pm 2	10	99 \pm 2	8	107 \pm 5	8	110 \pm 2	8	100 \pm 2	10	107 \pm 3*	10	107 \pm 3*	10	107 \pm 3*	10
RVC (U)	82 \pm 10	7	81 \pm 11	7	88 \pm 8	7	87 \pm 8	9	85 \pm 7	10	89 \pm 8	10	76 \pm 8	10	79 \pm 6	8	103 \pm 16	7	83 \pm 12*	7	81 \pm 12	6	85 \pm 7	6	85 \pm 7	6	85 \pm 7	6
MVC (U)	78 \pm 4	7	71 \pm 6	7	86 \pm 9	7	82 \pm 9	9	81 \pm 8	9	99 \pm 11	9	74 \pm 11	9	71 \pm 13	7	71 \pm 6	8	67 \pm 8	8	101 \pm 12	9	82 \pm 11	9	82 \pm 11	9	82 \pm 11	9
HVC (U)	34 \pm 6	7	33 \pm 6	7	47 \pm 3	7	43 \pm 5	9	51 \pm 5	10	47 \pm 4	10	49 \pm 5	10	49 \pm 4	7	35 \pm 7	7	31 \pm 6	7	54 \pm 9	9	47 \pm 7	9	47 \pm 7	9	47 \pm 7	9

Note: Values are mean \pm SEM. Units of vascular conductance (VC) are kHz. mmHg⁻¹ \times 10³. n = 6–10 per group.

Wilcoxon matched-pairs signed-rank test. * p < .05 versus corresponding vehicle group.

Abbreviations: HR, heart rate; HVC, hindquarters vascular conductance; MAP, mean arterial pressure; MVC, mesenteric vascular conductance; RVC, renal vascular conductance; U, units.

[†]In some instances agonist administration was delayed past 90 min in the case of movement to allow the rat to settle.

5 s) followed by 90 min intravenous infusion (0.4 ml.h⁻¹) on day 1 and an ICI 118,551 intravenous bolus (2.0 mg.kg⁻¹) (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (1.0 mg.kg⁻¹.h⁻¹) (0.4 ml.h⁻¹) on day 3. **Group 2** received ICI 118,551 (2.0 mg.kg⁻¹, 0.1 ml i.v. bolus; 1.0 mg.kg⁻¹.h⁻¹, 0.4 ml.h⁻¹ i.v. infusion) on day 1 and vehicle (0.1 ml i.v. bolus; 0.4 ml.h⁻¹ i.v. infusion) on day 3. Approximately 90 min after the initial bolus of vehicle or ICI 118,551, all groups received intravenous infusions (0.1 ml.min⁻¹) of BAY 60-6583 (4.0 (low), 13.3 (mid), and 40.0 (high) µg.kg⁻¹ min⁻¹). Each dose was infused for 3 min. Hemodynamic recordings were made for a further 4 h following the completion of the BAY 60-6583 intravenous infusion period.

2.4.6 | Study 6: The effect of β antagonist propranolol (1.0 mg.kg⁻¹ bolus; 0.5 mg.kg⁻¹.h⁻¹, 90 min infusion) on the hemodynamic profile of A_{2B} agonist BAY 60-6583

Ten animals were used to assess the cardiovascular responses to BAY 60-6583 in the presence or absence of propranolol. After a period of baseline recordings, rats were randomized into two groups. **Group 1** received vehicle intravenous bolus (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (0.4 ml.h⁻¹) on day 1 and a propranolol intravenous bolus (1 mg.kg⁻¹) (0.1 ml provided over 5 s) followed by 90 min intravenous infusion (0.5 mg.kg⁻¹.h⁻¹) (0.4 ml.h⁻¹) on day 3. **Group 2** received propranolol (1.0 mg.kg⁻¹, 0.1 ml i.v. bolus; 0.5 mg.kg⁻¹.h⁻¹, 0.4 ml.h⁻¹ i.v. infusion) on day 1 and vehicle (0.1 ml i.v. bolus; 0.4 ml.h⁻¹ i.v. infusion) on day 3. Approximately 90 min after the initial bolus of vehicle or propranolol, all groups received intravenous infusions (0.1 ml.min⁻¹) of BAY 60-6583 (4.0 (low), 13.3 (mid), and 40.0 (high) µg.kg⁻¹ min⁻¹). Each dose was infused for 3 min. Hemodynamic recordings were made for a further 4 h following the completion of the BAY 60-6583 intravenous infusion period.

2.5 | NanoBRET ligand binding studies

The evaluation of the binding of β-adrenoceptor ligands to rat Nanoluciferase-tagged adenosine A_{2A} and A_{2B} receptors expressed in HEK293T cells was performed as previously described in Cooper et al. 2022.¹⁰ Briefly, competition binding experiments were performed with 50 nM CA200645, in the presence or absence of increasing concentrations of ICI 118,551, CGP 20712A or propranolol, in HEPES-buffered saline solution (HBSS; 147 mM NaCl, 5 mM KCl, 1.3 mM CaCl₂, 1 mM MgSO₄, 10 mM HEPES, 2 mM sodium pyruvate, 1.43 mM NaHCO₃, 10 mM d-glucose, pH 7.45, supplemented with 0.1% BSA). Cells were incubated with ligand for 2 h at 37°C. Furimazine was then added to each well to give a final concentration of 10 µM. The cells were incubated for a further 5 min at 37°C. A PHERAstar FS plate reader (BMGLabtech) was used to measure the resulting bioluminescence resonance energy transfer (BRET) using filtered light emissions at 460 nm (80 nm bandpass) and >610 nm (longpass) at 37°C. The

ratio between the >610 nm emission and the 460 nm emission provided the raw BRET data for each experiment.

2.6 | Data analysis

All in vivo data were collected and analysed using IdeeQ software (Maastricht Instruments, Maastricht University, NL). For all experiments, time-averaged data are shown as changes from baseline [HR (beats.min⁻¹); MAP (mmHg); VC (%)]. Statistical comparisons between groups of animals were performed on the integrated changes over specified time periods. A Friedman's nonparametric repeated-measures analysis of variance was used for within-group comparisons, and a Wilcoxon rank-sum test for integrated area under or above curve analysis was used for comparisons between groups. A Wilcoxon test was also performed for comparisons between groups at specific time points. Vascular conductances were calculated from the MAP and Doppler shift (flow) data. A value of $p < .05$ was considered significant.

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY²⁸ and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.²⁹

3 | RESULTS

Treatment of rats with the selective β₁-adrenoceptor antagonist CGP 20712A confirmed that there was a significant basal sympathetic tone involving β₁-adrenoceptors in maintaining HR, and showed modest increases in renal and hindquarters vascular conductance (Figure 1). In contrast, the selective β₂-adrenoceptor antagonist ICI 118,551 produced a short-lived transient decrease in HR and small decreases in vascular conductance in all three vascular beds (Figure 1). The data obtained with propranolol were consistent with antagonism of β₁-adrenoceptors in the heart and β₂-adrenoceptor antagonism in each vascular bed (Figure 1). Baseline cardiovascular variables for the combined results of studies before the administration of CGP 20712A, ICI 118,551, or propranolol are summarized in Table 1 and correspond to the baseline values shown in Figure 1. Baseline cardiovascular variables before the administration of CGP 20712A, ICI 118,551 or propranolol, adenosine receptor agonists, or their corresponding vehicle controls are shown in Table 2; these baseline values correspond to the baseline values shown in Figures 2-7.

3.1 | Effect of β-adrenoceptor antagonists on the hemodynamic profile of the A_{2A} agonist CGS 21680

Consistent with our previous observations,¹⁰ the selective A_{2A} agonist CGS 21680 produced a marked increase in vascular conductance in the hindquarters of conscious, freely moving rats without a

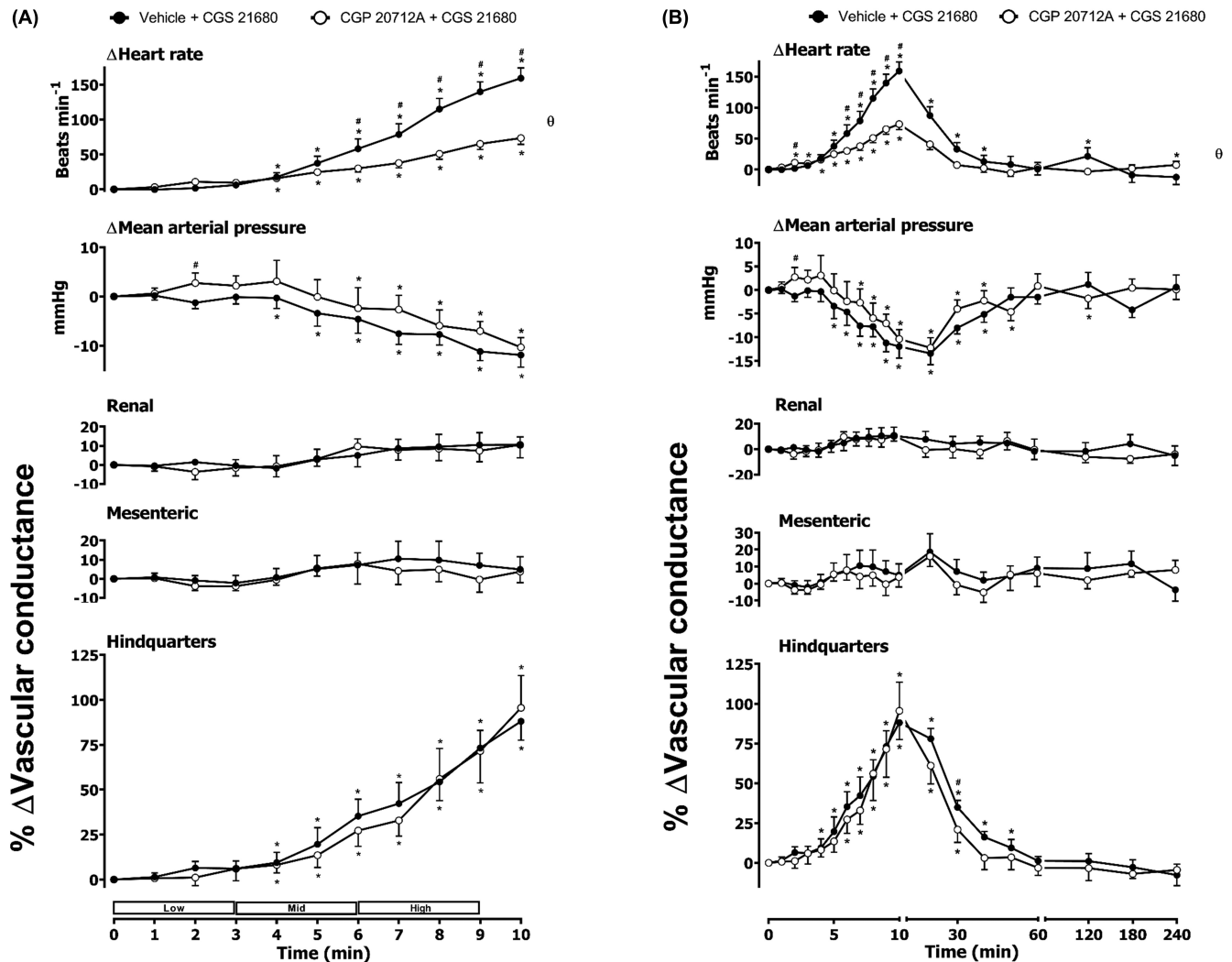


FIGURE 2 Cardiovascular responses to CGS 21680 in the presence or absence of CGP 20712A in conscious, freely moving rats. Rats were dosed with CGP 20712A (0.1 ml bolus dose of $200 \mu\text{g}\cdot\text{kg}^{-1}$, followed by a 90 min infusion of $100 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ i.v., $n = 8$) or vehicle (0.1 ml bolus and a 90 min infusion of 5% propylene glycol, 2% Tween 80 in sterile saline, $n = 8$) as described in the methods. Following the 90 min infusion, all animals received an infusion of CGS 21680 ($0.1, 0.3$ and $1.0 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; each dose infused (i.v.) over 3 min). The time courses show (A) the treatment period and (B) the treatment period plus the extended 4 h recording period. Data points are mean and vertical bars represent SEM. * $p < .05$ versus baseline (Friedman's test). A Wilcoxon signed-rank test was conducted between treated and vehicle control groups for a comparison of area under/over the curve ($\theta p < .05$) and to determine differences at each time point ($\# p < .05$, Wilcoxon T-test equivalent).

significant effect on blood flow in the renal and mesenteric circulations (Figure 2). This change in hindquarters VC was accompanied by a significant increase in HR and a fall in MAP. The addition of the β_1 adrenoceptor-selective antagonist CGP 20712A, 90 min prior to the increasing concentrations of the A_{2A} agonist CGS 21680, significantly attenuated the A_{2A} agonist-induced increase in HR ($p < .05$; Figure 2). However, no significant difference was observed in MAP, RVC, MVC, and HVC between vehicle-pretreated rats and CGP 20712A-pretreated rats (Figure 2). This dose of CGP 20712A was selected as it has previously been shown to produce a highly selective antagonism of cardiac β_1 -adrenoceptors in vivo in the rat.^{30,31} Selective antagonism of β_2 -adrenoceptors with ICI 118,551,^{30,31} however, produced a small but significant increase in the RVC

response mediated by CGS 21680, but did not alter HVC, HR, or MAP (Figure 3). Pretreatment with the non-selective β -blocker propranolol produced a marked ($p < .05$) decrease in HR with no significant change in MAP (Figure 4). Propranolol did, however, produce a small reduction ($p < .05$) in the hindquarters vasodilatation (Figure 4).

3.2 | Effect of β -adrenoceptor antagonists on the hemodynamic profile of the A_{2B} agonist BAY 60-6583

As noted previously,¹⁰ the A_{2B} agonist BAY 60-6583 produced a large increase in HR as well as significant increases in RVC and

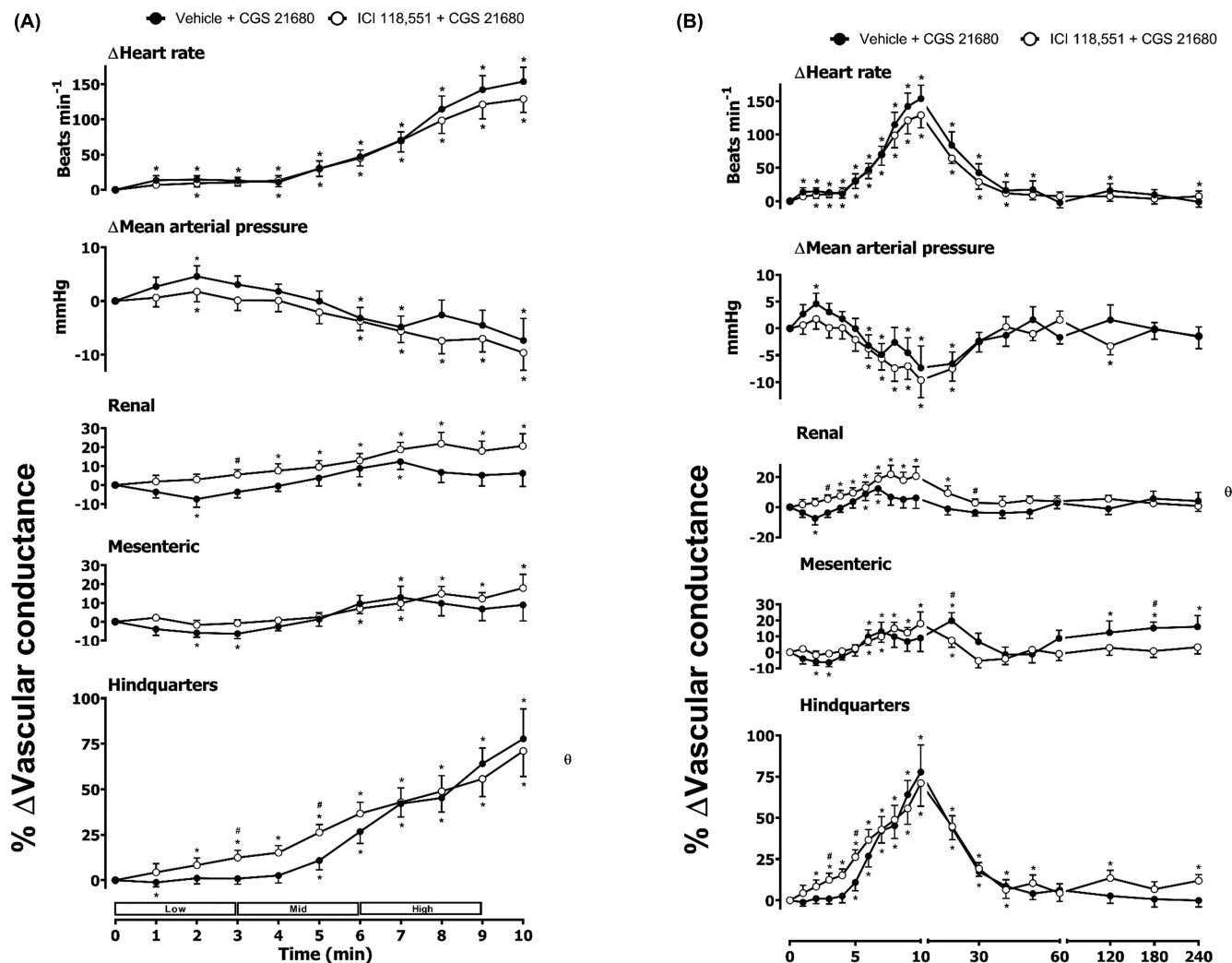


FIGURE 3 Cardiovascular responses to CGS 21680 in the presence or absence of ICI 118,551 in conscious, freely moving rats. Rats were dosed with ICI 118,551 (0.1 ml bolus dose of 2.0 mg.kg⁻¹, followed by a 90 min infusion of 1.0 mg.kg⁻¹.h⁻¹ i.v., *n* = 9) or vehicle (0.1 ml bolus and a 90 min infusion of 5% propylene glycol, 2% Tween 80 in sterile saline, *n* = 9) as described in the methods. Following the 90 min infusion, all animals received an infusion of CGS 21680 (0.1, 0.3, and 1.0 μg.kg⁻¹.min⁻¹; each dose infused (i.v.) over 3 min). The time courses show (A) the treatment period and (B) the treatment period plus the extended 4 h recording period. Data points are mean and vertical bars represent SEM. **p* < .05 versus baseline (Friedman's test). A Wilcoxon signed-rank test was conducted between treated and vehicle control groups for a comparison of area under/over the curve (θ *p* < .05) and to determine differences at each time point (# *p* < .05, Wilcoxon T-test equivalent).

MVC (Figure 5). There was no significant effect of BAY 60-6583 on MAP or hindquarters blood flow (Figure 5). Pre-treatment with the β₁-adrenoceptor-selective antagonist CGP 20712A produced a significant attenuation (*p* < .05) of the heart rate response to BAY 60-6583 without any change in the vasodilatation induced in the renal and mesenteric circulations (Figure 5). The β₂-adrenoceptor selective antagonist ICI 118,551 significantly increased the HR, RVC, and MVC responses induced by the A_{2B} agonist (Figure 6). Antagonism of both β₁- and β₂-adrenoceptors produced a marked decrease in the HR response to BAY 60-6583 and a small reduction in MAP in the presence of both propranolol and BAY 60-6583 (Figure 7). Propranolol did not, however, attenuate the vasodilatation induced by the

selective A_{2B}-receptor agonist in the renal and mesenteric circulations.

3.3 | NanoBRET ligand binding studies

To verify that the β-adrenoceptor ligands do not directly bind to either of the two A₂ adenosine receptors, we also performed NanoBRET ligand binding studies utilizing rat N-terminal Nanoluciferase-tagged A_{2A} and A_{2B} receptors, as described previously.¹⁰ CGP 20712A, ICI 118,551, and propranolol had no effect on the specific binding of the non-selective fluorescent ligand CA200645 (50 nM) to either rat receptor expressed in HEK293T cells (*n* = 5) at concentrations up to

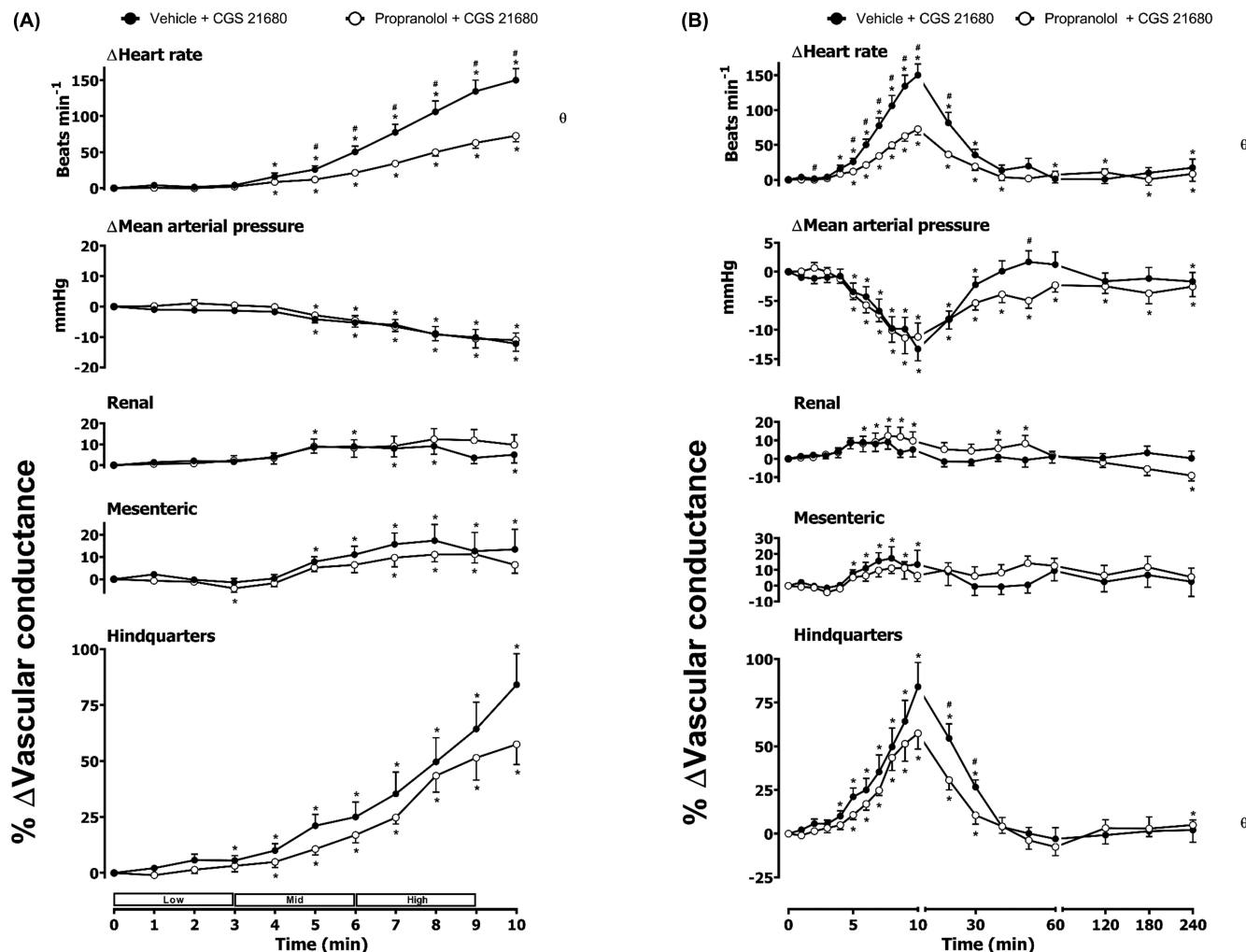


FIGURE 4 Cardiovascular responses to CGS 21680 in the presence or absence of propranolol in conscious, freely moving rats. Rats were dosed with propranolol (0.1 ml bolus dose of $1 \text{ mg} \cdot \text{kg}^{-1}$, followed by a 90 min infusion of $0.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ i.v., $n = 10$) or vehicle (0.1 ml bolus and a 90 min infusion of 5% propylene glycol, 2% Tween 80 in sterile saline, $n = 10$) as described in the methods. Following the 90 min infusion, all animals received an infusion of CGS 21680 (0.1, 0.3, and $1.0 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$; each dose infused (i.v.) over 3 min). The time courses show (A) the treatment period and (B) the treatment period plus the extended 4 h recording period. Data points are mean and vertical bars represent SEM. * $p < .05$ versus baseline (Friedman's test). A Wilcoxon signed-rank test was conducted between treated and vehicle control groups for a comparison of area under/over the curve ($\theta p < .05$) and to determine differences at each time point ($\# p < .05$, Wilcoxon T-test equivalent).

$100 \mu\text{M}$, suggesting that none of the β -adrenoceptor ligands tested directly bind to either of the two rat A_2 receptors.

4 | DISCUSSION

Consistent with our previous observations,¹⁰ the selective A_{2A} agonist CGS 21680 produced a marked increase in HR that was associated with a fall in MAP in conscious rats. A similar effect has been reported with the A_{2A} receptor agonist regadenoson.³² These authors went on to suggest that the increase in HR was mediated by a direct A_{2A} -receptor-mediated stimulation of the sympathetic nervous system since the HR response, but not the fall in MAP, was attenuated by the β -blocker metoprolol.³² This result suggests that a baroreflex-mediated increase in HR is not the sole reason for the

tachycardia observed with CGS 21680.³² In the present study, we therefore investigated the effect of selective β_1 - and β_2 - selective antagonists on the different cardiovascular responses to the A_{2A} -selective agonist CGS 21680 and the A_{2B} -selective agonist BAY 60-6583 to determine the extent to which they are secondary to sympathetic excitation (Figure 8).

To do this, we chose a dosage regimen with CGP 20712A and ICI 118,551 that produced highly selective antagonism in vivo of β_1 - and β_2 - adrenoceptor cardiovascular responses respectively.^{30,31} The selective β_1 -adrenoceptor antagonist CGP 20712A significantly attenuated the HR response to CGS 21680 without changing the fall MAP, consistent with previous findings obtained using regadenoson.³² These data suggest that a direct activation of the sympathetic nervous system is indeed induced by A_{2A} -receptor stimulation. It was therefore important to determine what effect CGP 20712A or ICI

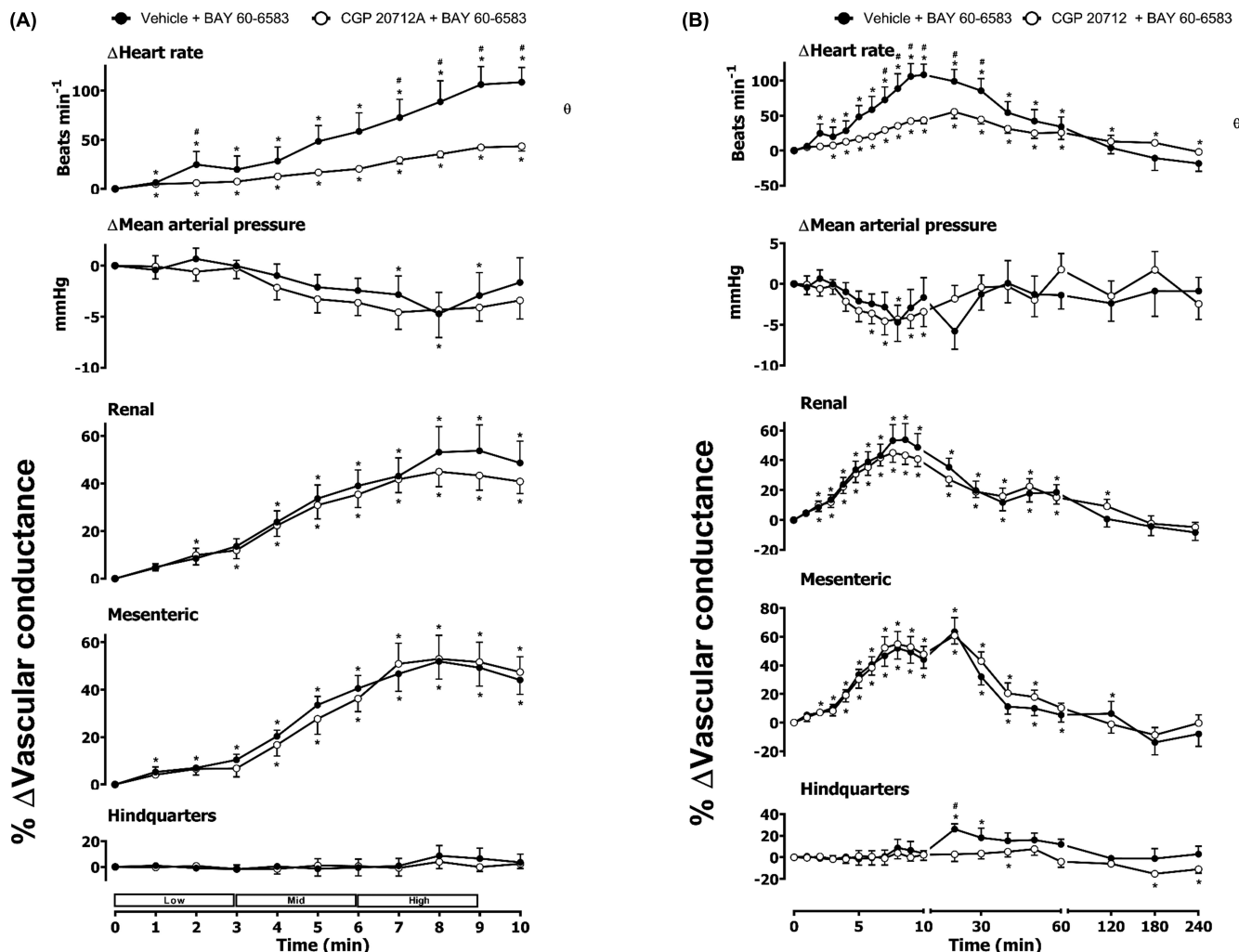


FIGURE 5 Cardiovascular responses to BAY 60-6583 in the presence or absence of CGP 20712A in conscious, freely moving rats. Rats were dosed with CGP 20712A (0.1 ml bolus dose of 200 $\mu\text{g}\cdot\text{kg}^{-1}$, followed by a 90 min infusion of 100 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ i.v., $n = 8$) or vehicle (0.1 ml bolus and a 90 min infusion of 5% propylene glycol, 2% Tween 80 in sterile saline, $n = 8$) as described in the methods. Following the 90 min infusion, all animals received an infusion of BAY 60-6583 (4.0, 13.3, and 40.0 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; each dose infused (i.v.) over 3 min. The time courses show (A) the treatment period and (B) the treatment period plus the extended 4 h recording period. Data points are mean and vertical bars represent SEM. * $p < 0.05$ versus baseline (Friedman's test). A Wilcoxon signed-rank test was conducted between treated and vehicle control groups for a comparison of area under/over the curve ($\theta p < .05$) and to determine differences at each time point (# $p < .05$, Wilcoxon T-test equivalent).

118,551 treatment would have on the regionally selective increase in vascular conductance in the hindquarters observed with CGS 21680 in conscious, freely moving rats. However, neither CGP 20712A nor ICI 118,551 had any effect on HVC. Furthermore, ICI 118,551 did not change the increase in HR or the fall in MAP induced by this A_{2A} -selective agonist. As expected, the non-selective β -blocker propranolol reduced the tachycardia induced by CGS 21680 without affecting MAP responses. However, propranolol did produce a small attenuation of the hindquarters vasodilatation, which may be related to its off-target actions on other receptors, such as the 5-HT_{1B} receptor.³³

In the case of the A_{2B} -receptor selective agonist BAY 60-6583, the profile of cardiovascular responses was quite different to that obtained with CGS 21680.¹⁰ Although BAY 60-6583 produced a

large increase in heart rate, this was not accompanied by a change in MAP consistent with the tachycardia being due to either a direct effect of A_{2B} -receptor activation in the heart or due to a similar excitation of the sympathetic nervous system described above for A_{2A} -receptors. In keeping with the latter hypothesis, CGP 20712A significantly attenuated the HR response to BAY 60-6583. In contrast with A_{2A} -receptor stimulation, A_{2B} -receptor activation produced increases in vascular conductance in the renal and mesenteric vascular beds without a change in the hindquarters. These changes in vascular conductance were increased by treatment with ICI 118,551, but were not altered by CGP 20712A. ICI 118,551 treatment also produced a small significant increase in the HR response to BAY 60-6583. It was notable that in the absence of adenosine receptor agonist treatment, ICI 118,551 produced a significant transient fall

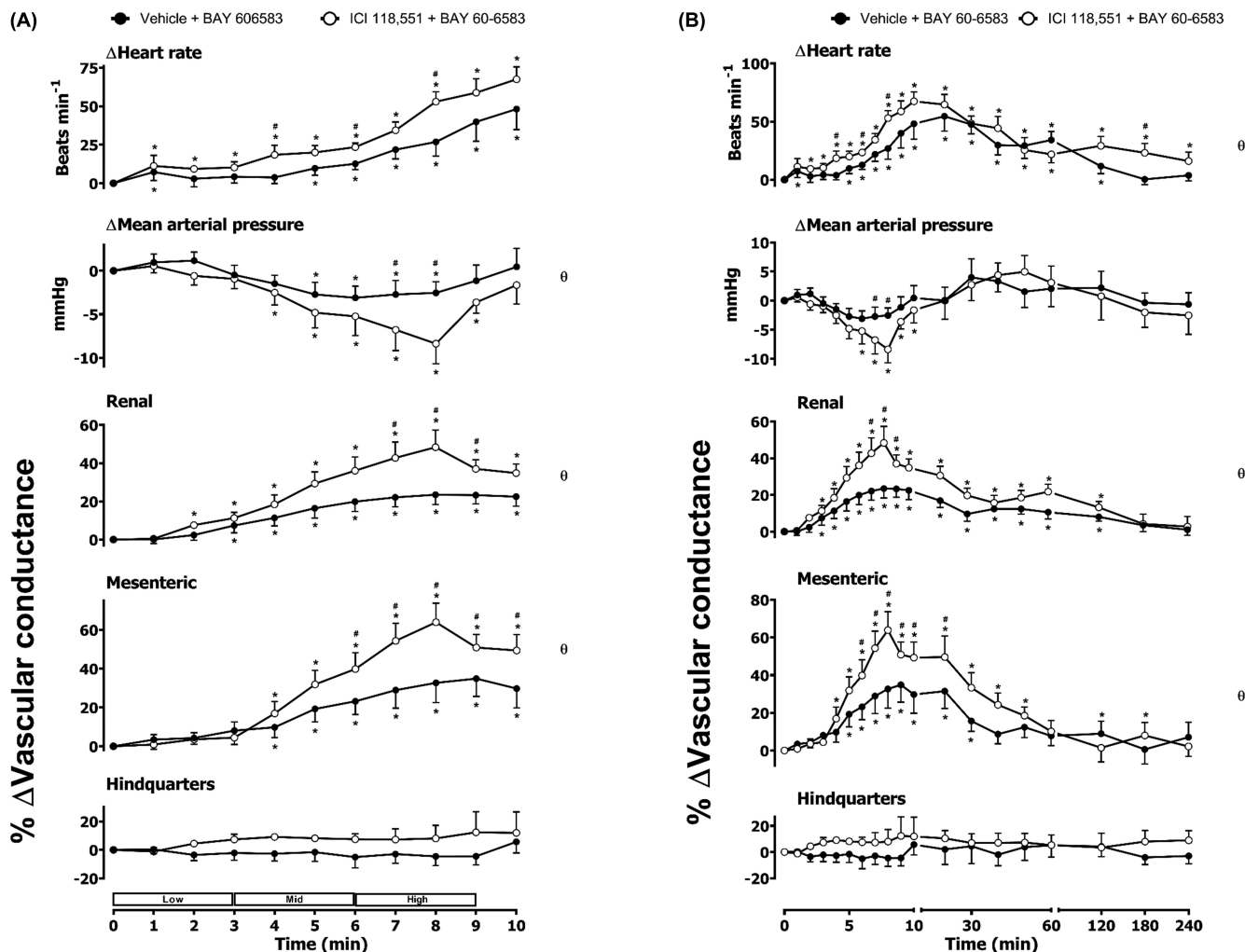


FIGURE 6 Cardiovascular responses to BAY 60-6583 in the presence or absence of ICI 118,551 in conscious, freely moving rats. Rats were dosed with ICI 118,551 (0.1 ml bolus dose of 2.0 mg.kg⁻¹, followed by a 90 min infusion of 1.0 mg.kg⁻¹.h⁻¹ i.v., *n* = 8) or vehicle (0.1 ml bolus and a 90 min infusion of 5% propylene glycol, 2% Tween 80 in sterile saline, *n* = 8) as described in the methods. Following the 90 min infusion, all animals received an infusion of BAY 60-6583 (4.0, 13.3, and 40.0 μg.kg⁻¹.min⁻¹; each dose infused (i.v.) over 3 min). The time courses show (A) the treatment period and (B) the treatment period plus the extended 4 h recording period. Data points are mean and vertical bars represent SEM. **p* < 0.05 versus baseline (Friedman's test). A Wilcoxon signed-rank test was conducted between treated and vehicle control groups for a comparison of area under/over the curve (θ *p* < .05) and to determine differences at each time point (# *p* < .05, Wilcoxon T-test equivalent).

in HR and more prolonged decreased in both RVC and MVC. This is consistent with a significant β_2 -adrenoceptor-mediated sympathetic tone under basal conditions that is removed by ICI 118,551. It is therefore likely that the enhanced effects of BAY 60-6583 on HR, RVC, and MVC in the presence of ICI 118,551 is a consequence of the direct A_{2B}-receptor responses being able to achieve maximum responses from a lower baseline signal. This is also seen to some extent with CGS 21680 on the RVC where, in the presence of ICI 118,551 a small significant vasodilation is revealed in the presence of the β_2 -adrenoceptor antagonist.

Taken together, these data suggest that the regionally selective effects of A_{2A} and A_{2B} receptor activation on vascular conductance are the result of direct activation of receptors in the respective vascular beds and are not secondary to activation of the sympathetic nervous system. In contrast, the tachycardia

induced by both receptor subtypes seems to involve a component of direct sympathetic activation leading to stimulation of β_1 -adrenoceptors in the heart. Adenosine has been shown to activate afferent nerve terminals in the kidney and heart leading to sympathetic activation,^{34,35} but the exact mechanisms involved remains to be established. However, in the presence of a high dose of a selective β_1 -adrenoceptor antagonist, which produces a marked and selective antagonism in β_1 -adrenoceptor responses to isoprenaline in the heart,³⁰ both A_{2A} and A_{2B} agonists still caused a small increase in HR, indicative of some direct chronotropic effect at the level of the heart.

Both A_{2A} and A_{2B} receptors are expressed in the heart and coronary arteries.^{36,37} Indeed, the A_{2A} selective agonist regadenoson has been developed clinically as a coronary vasodilator for myocardial perfusion imaging.³⁸ In atrial samples taken from patients with atrial fibrillation,

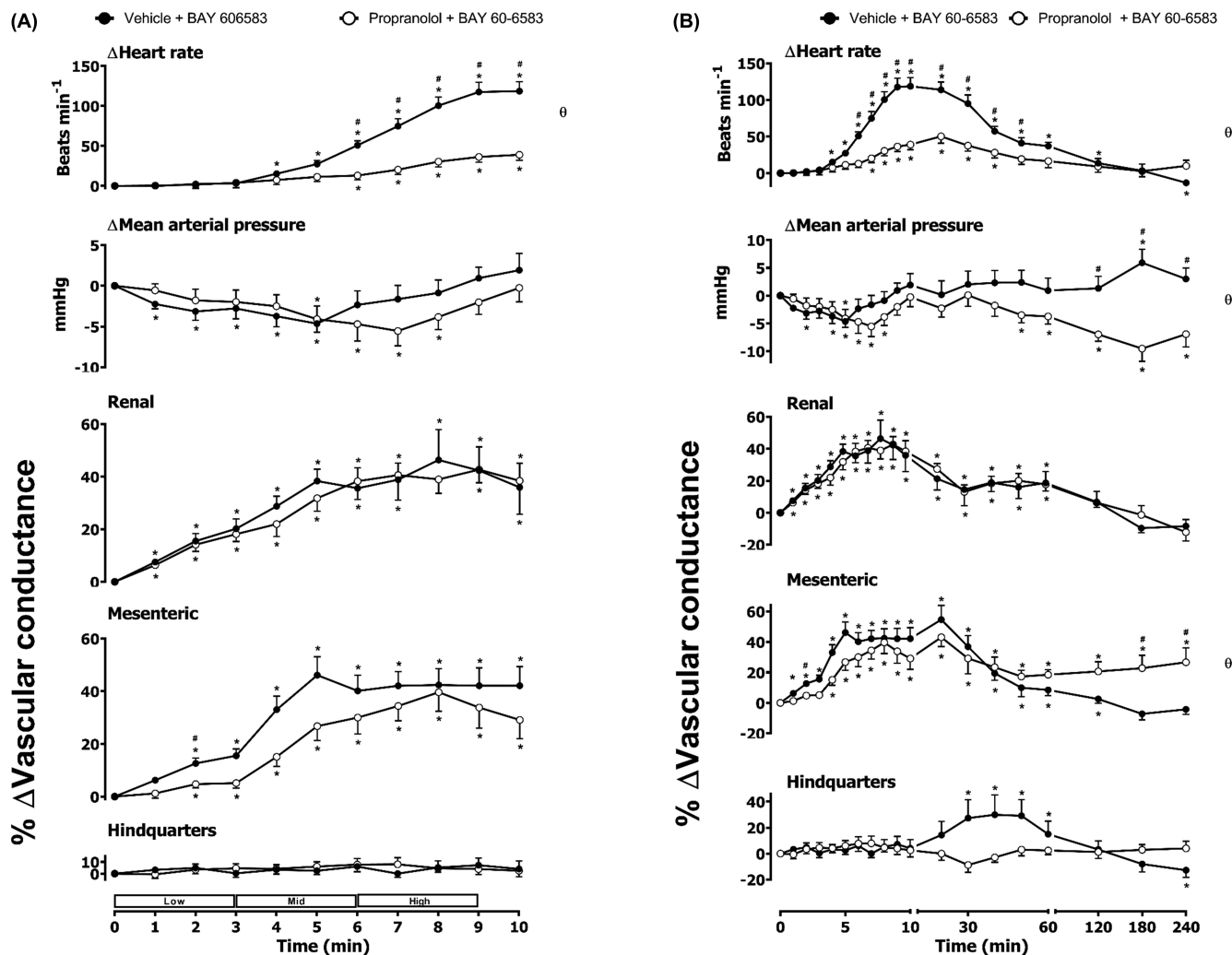


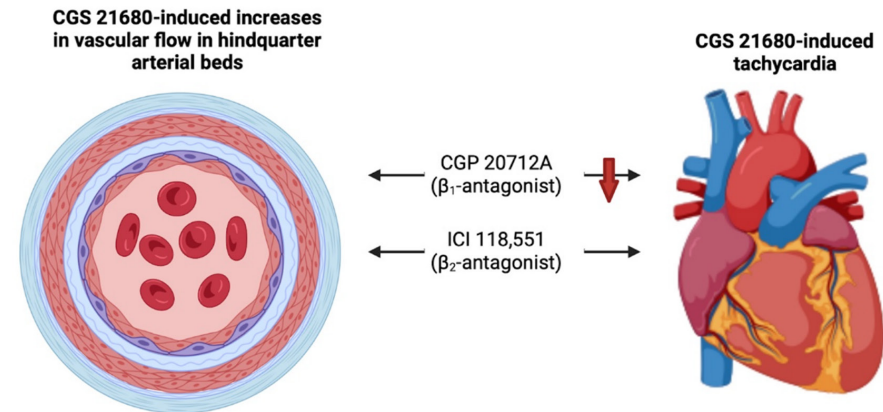
FIGURE 7 Cardiovascular responses to BAY 60-6583 in the presence or absence of propranolol in conscious, freely moving rats. Rats were dosed with propranolol (0.1 ml bolus dose of 1 mg.kg⁻¹, followed by a 90 min infusion of 0.5 mg.kg⁻¹.h⁻¹ i.v., n = 10) or vehicle (0.1 ml bolus and a 90 min infusion of 5% propylene glycol, 2% Tween 80 in sterile saline, n = 10) as described in the methods. Following the 90 min infusion, all animals received an infusion of BAY 60-6583 (4.0, 13.3, and 40.0 μg.kg⁻¹.min⁻¹; each dose infused (i.v.) over 3 min. The time courses show (A) the treatment period and (B) the treatment period plus the extended 4 h recording period. Data points are mean and vertical bars represent SEM. *p < .05 versus baseline (Friedman's test). A Wilcoxon signed-rank test was conducted between treated and vehicle control groups for a comparison of area under/over the curve (θ p < .05) and to determine differences at each time point (# p < .05, Wilcoxon T-test equivalent).

an increase in expression of A_{2A} receptors has been reported, which is linked to abnormal calcium release from the sarcoplasmic reticulum.^{39,40} A reduction in the expression of ventricular A_{2A}-receptors has also been observed in patients with chronic heart failure.⁴¹ Evidence for a direct role of A_{2A} receptors on cardiac contractility, however, remains unclear with contradictory reports of the effect of CGS 21680 on contractile activity and cyclic AMP accumulation in rat ventricular myocytes and isolated rat heart preparations.⁴²⁻⁴⁵

BAY 60-6583 has previously been shown to be cardioprotective and to reduce reperfusion injury and myocardial infarct size in isolated rat hearts.⁴⁶⁻⁴⁸ A_{2B} receptor stimulation also inhibits proliferation and collagen synthesis in isolated cardiac fibroblasts.⁴⁹⁻⁵¹ Furthermore, treatment with the A_{2B}-receptor antagonist GS-6201 has been shown to improve cardiac function by preventing the

remodeling and fibrogenesis that occurs following A_{2B} receptor activation.⁵² Interestingly, tissue-specific knockout of A_{2B} receptors from both cardiomyocytes and vascular endothelial cells showed that A_{2B}-receptors were critical for ischemia-reperfusion injury-elicited cardioprotection, but reperfusion injury was also increased if A_{2B}-receptor signaling was knocked out from inflammatory cells.⁵³ Selective activation of A_{2B}-receptors has also been demonstrated to play an important role in both renal and mesenteric ischemic reperfusion injury by improving capillary flow and function.^{54,55} The fact that BAY 60-6583 can increase blood flow in both the renal and mesenteric circulation without producing a change in MAP¹⁰ suggests that use of selective A_{2B}-receptor agonists may be a promising approach for the treatment of acute kidney injury and mesenteric ischemia. Although A_{2B}-receptor agonism resulted in attenuation of ischemic kidney injury in a murine

(A)

CGS 21680 (A_{2A} Agonist)

(B)

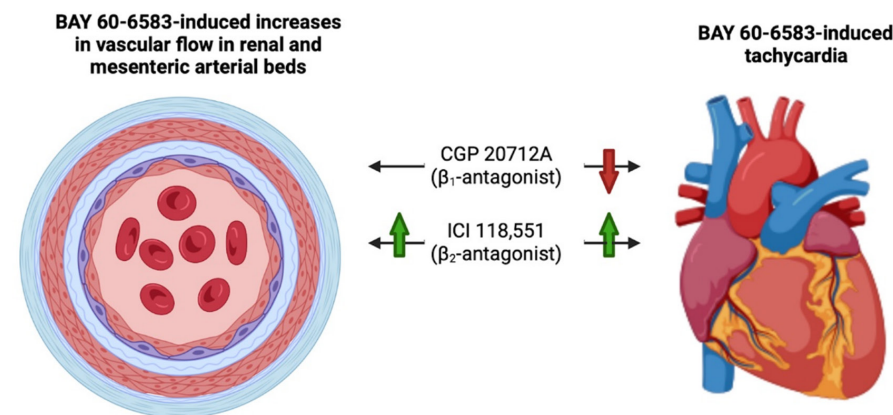
BAY 60-6583 (A_{2B} Agonist)

FIGURE 8 Summary of the key experimental findings. The impact of β_1 -antagonist, CGP 20712A and β_2 -antagonist, ICI 118,551 on the heart rate and vascular conductance profiles of (A) A_{2A} agonist, CGS 21680, and (B) A_{2B} agonist, BAY 60-6583 (created with Biorender.com).

model⁵⁴ and elicited renal vasodilation in conscious rats,¹⁰ the molecular mechanisms underlying A_{2B} receptor-mediated renovascular protection are yet to be fully defined. As a result, addressing the specific role of A_{2B} signaling in renal ischemia-reperfusion injury might be crucial to define the potential therapeutic uses of A_{2B} receptor agonists in the context of renal injury.

In summary, the present study has confirmed that the tachycardia induced by the selective A_{2A} -receptor agonist CGS 21680 is partly due to an activation of the sympathetic nervous system, and can be readily attenuated by the selective β_1 -adrenoceptor antagonist CGP 20712A. In contrast, neither β_1 - nor β_2 -selective antagonists had any significant effect on the fall in MAP or the increase in vascular conductance in the hindquarters vascular bed induced by CGS 21680. Similarly, the regionally selective vasodilator effects of the A_{2B} -selective agonist BAY 60-6583 in the renal and mesenteric circulations were not secondary to indirect sympathetic activation of β_1 - or β_2 -adrenoceptors. Importantly, we have also shown that the large increase in HR produced by BAY 60-6583 can be markedly attenuated by selective β_1 -adrenoceptor antagonists without significant effect on the changes

in vascular conductance. Furthermore, infusion of BAY 60-6583 did not alter mean arterial pressure (in the presence or absence of β_1 -adrenoceptor blockade) (Figure 8). These data suggest that the conjoint use of an A_{2B} -selective agonist and a β -blocker might be an effective way to achieve a beneficial effect in the treatment of acute kidney injury or mesenteric ischemia.

4.1 | Study limitations

Although in this rodent model our findings indicate that selective activation of A_{2A} and A_{2B} -receptors produces regionally selective vasodilations and that specific A_{2B} -receptor vascular responses can be enhanced by β_2 -adrenoceptor antagonism (Figure 8), it is important to note that ligand affinity and potency at adenosine receptors and other GPCRs can differ significantly between species and must be taken into account when considering the implications of this research.⁵⁶⁻⁵⁸ Furthermore, the acute studies presented here only give an indication of the short-term impact of the ligands

investigated. All four adenosine receptor subtypes are known to undergo agonist-induced desensitization, internalization, and cellular trafficking, the effect of which may not be seen in these short-term *in vivo* studies.^{59,60} Therefore, future studies should include longer term dosing regimens and observations, in addition to clinical studies, to confirm that the conclusions presented here translate to the human condition.

AUTHOR CONTRIBUTIONS

Participated in research design: SLC, JW, SJH, ESW, *Conducted experiments:* ESW, PP, SLC, *Performed data analysis:* ESW, PP, SLC, JW, *Wrote or contributed to the writing of the manuscript:* SJH, SLC, PP, JW, ESW.

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DISCLOSURES

The authors declare no conflicts of interest.

ETHICS STATEMENT

All procedures were performed with approval of the University of Nottingham Animal Welfare and Ethical Review Board and performed in line with the Animals (Scientific Procedures) Act (1986), under UK Home Office approved Project License and Personal License authority. 53 rats were used during this study, and all animal experiments are reported in compliance with the ARRIVE guidelines²⁵ and the editorial on reporting animal studies.²⁶

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Burnstock G. Purinergic signaling in the cardiovascular system. *Circ Res.* 2017;120(1):207-228.
2. Guieu R, Deharo J-C, Maille B, et al. Adenosine and the cardiovascular system: the good and the bad. *J Clin Med.* 2020;9(5):1366.
3. Headrick JP, Peart JN, Reichelt ME, Haseler LJ. Adenosine and its receptors in the heart: regulation, retaliation and adaptation. *Biochim Biophys Acta.* 2011;1808(5):1413-1428.
4. Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Müller CE. International union of basic and clinical pharmacology. LXXXI. nomenclature and classification of adenosine receptors—an update. *Pharmacol Rev.* 2011;63(1):1-34.
5. Headrick JP, Ashton KJ, Rosemeyer RB, Peart JN. Cardiovascular adenosine receptors: expression, actions and interactions. *Pharmacol Ther.* 2013;140(1):92-111.
6. Johnston-Cox HA, Koupenova M, Ravid K. A2 adenosine receptors and vascular pathologies. *Arterioscler Thromb Vasc Biol.* 2012;32(4):870-878.
7. Borea PA, Gessi S, Merighi S, Vincenzi F, Varani K. Pharmacology of adenosine receptors: the state of the art. *Physiol Rev.* 2018;98(3):1591-1625.
8. Bahreyni A, Avan A, Shabani M, et al. Therapeutic potential of A2 adenosine receptor pharmacological regulators in the treatment of cardiovascular diseases, recent progress, and prospective. *J Cell Physiol.* 2019;234(2):1295-1299.
9. Thomas T, St Lambert JH, Dashwood MR, Spyer KM. Localization and action of adenosine A2a receptors in regions of the brainstem important in cardiovascular control. *Neuroscience.* 2000;95(2):513-518.
10. Cooper SL, Wragg ES, Pannucci P, Soave M, Hill SJ, Woolard J. Regionally selective cardiovascular responses to adenosine A2A and A2B receptor activation. *FASEB J.* 2022;36(4):e22214.
11. Sheth S, Brito R, Mukherjee D, Rybak LP, Ramkumar V. Adenosine receptors: expression, function and regulation. *Int J Mol Sci.* 2014;15(2):2024-2052.
12. Paganelli F, Gaudry M, Ruf J, Guieu R. Recent advances in the role of the adenosinergic system in coronary artery disease. *Cardiovasc Res.* 2021;117(5):1284-1294.
13. Alencar AKN, Montes GC, Barreiro EJ, Sudo RT, Zapata-Sudo G. Adenosine receptors as drug targets for treatment of pulmonary arterial hypertension. *Front Pharmacol.* 2017;8:858.
14. Yap SC, Lee HT. Adenosine and protection from acute kidney injury. *Curr Opin Nephrol Hypertens.* 2012;21(1):24-32.
15. Jamwal S, Mittal A, Kumar P, Alhayani DM, Al-Aboudi A. Therapeutic potential of agonists and antagonists of A1, A2a, A2b and A3 adenosine receptors. *Curr Pharm Des.* 2019;25(26):2892-2905.
16. Sved AF. Blood pressure: baroreceptors. In: Squire LR, ed. *Encyclopedia of neuroscience.* Academic Press; 2009:259-264.
17. Kougias P, Weakley SM, Yao Q, Lin PH, Chen C. Arterial baroreceptors in the management of systemic hypertension. *Med Sci Monit.* 2010;16(1):RA1-RA8.
18. Andresen MC. Cardiovascular Integration in the Nucleus of the Solitary Tract. In: Dun NJ, Machado BH, Pilowsky PM, eds. *Neural mechanisms of cardiovascular regulation.* Springer; 2004:59-80.
19. El-Mas MM, El-Gowilly SM, Fouda MA, Saad EI. Role of adenosine A2A receptor signaling in the nicotine-evoked attenuation of reflex cardiac sympathetic control. *Toxicol Appl Pharmacol.* 2011;254(3):229-237.
20. Lee TK, Koh HC. Involvement of NO and KATP channel in adenosine A2B receptors induced cardiovascular regulation in the posterior hypothalamus of rats. *J Cardiovasc Pharmacol.* 2009;53(2):167-172.
21. Charkoudian N, Rabbitts JA. Sympathetic neural mechanisms in human cardiovascular health and disease. *Mayo Clin Proc.* 2009;84(9):822-830.
22. Barki-Harrington L, Perrino C, Rockman HA. Network integration of the adrenergic system in cardiac hypertrophy. *Cardiovasc Res.* 2004;63(3):391-402.
23. Chruscinski A, Brede ME, Meinel L, Lohse MJ, Kobilka BK, Hein L. Differential distribution of beta-adrenergic receptor subtypes

- in blood vessels of knockout mice lacking beta(1)- or beta(2)-adrenergic receptors. *Mol Pharmacol*. 2001;60(5):955-962.
24. Ali DC, Naveed M, Gordon A, et al. β -Adrenergic receptor, an essential target in cardiovascular diseases. *Heart Fail Rev*. 2020;25(2):343-354.
 25. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol*. 2010;160(7):1577-1579.
 26. McGrath JC, Lilley E. Implementing guidelines on reporting research using animals (ARRIVE etc.): new requirements for publication in *BJP*. *Br J Pharmacol*. 2015;172(13):3189-3193.
 27. Carter JJ, Fretwell LV, Woolard J. Effects of 4 multitargeted receptor tyrosine kinase inhibitors on regional hemodynamics in conscious, freely moving rats. *FASEB J*. 2017;31(3):1193-1203.
 28. Harding SD, Sharman JL, Faccenda E, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res*. 2018;46(D1):D1091-d1106.
 29. Alexander SPH, Christopoulos A, Davenport AP, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2021/22: G protein-coupled receptors. *Br J Pharmacol*. 2021;178(S1):S27-S156.
 30. Baker JG, Gardiner SM, Woolard J, et al. Novel selective $\beta(1)$ -adrenoceptor antagonists for concomitant cardiovascular and respiratory disease. *FASEB J Off Publ Feder Am Soc Exp Biol*. 2017;31(7):3150-3166.
 31. Baker JG, Kemp P, March J, Fretwell L, Hill SJ, Gardiner SM. Predicting in vivo cardiovascular properties of β -blockers from cellular assays: a quantitative comparison of cellular and cardiovascular pharmacological responses. *Faseb J*. 2011;25(12):4486-4497.
 32. Dhalla AK, Wong MY, Wang WQ, Biaggioni I, Belardinelli L. Tachycardia caused by A2A adenosine receptor agonists is mediated by direct sympathoexcitation in awake rats. *J Pharmacol Exp Ther*. 2006;316(2):695-702.
 33. Pauwels PJ, Palmier C. Inhibition by 5-HT of forskolin-induced cAMP Formation in the renal opossum Epithelial cell line OK: Mediation by a 5-HT1B like receptor and antagonism by methiothepin. *Neuropharmacology*. 1994;33(1):67-75.
 34. Cox DA, Vita JA, Treasure CB, Fish RD, Selwyn AP, Ganz P. Reflex increase in blood pressure during the intracoronary administration of adenosine in man. *J Clin Invest*. 1989;84(2):592-596.
 35. Katholi RE, Whitlow PL, Hageman GR, Woods WT. Intrarenal adenosine produces hypertension by activating the sympathetic nervous system via the renal nerves in the dog. *J Hypertens*. 1984;2(4):349-359.
 36. Varani K, Laghi-Pasini F, Camurri A, et al. Changes of peripheral A2A adenosine receptors in chronic heart failure and cardiac transplantation. *Faseb J*. 2003;17(2):280-282.
 37. Gariboldi V, Vairo D, Guieu R, et al. Expressions of adenosine A(2A) receptors in coronary arteries and peripheral blood mononuclear cells are correlated in coronary artery disease patients. *Int J Cardiol*. 2017;230:427-431.
 38. Hage FG, Heo J, Franks B, et al. Differences in heart rate response to adenosine and regadenoson in patients with and without diabetes mellitus. *Am Heart J*. 2009;157(4):771-776.
 39. Llach A, Molina CE, Prat-Vidal C, et al. Abnormal calcium handling in atrial fibrillation is linked to up-regulation of adenosine A2A receptors. *Eur Heart J*. 2011;32(6):721-729.
 40. Molina CE, Llach A, Herraiz-Martínez A, et al. Prevention of adenosine A2A receptor activation diminishes beat-to-beat alternation in human atrial myocytes. *Basic Res Cardiol*. 2016;111(1):5.
 41. Asakura M, Asanuma H, Kim J, et al. Impact of adenosine receptor signaling and metabolism on pathophysiology in patients with chronic heart failure. *Hypertens Res*. 2007;30(9):781-787.
 42. Dobson JG Jr, Shea LG, Fenton RA. Adenosine A2A and beta-adrenergic calcium transient and contractile responses in rat ventricular myocytes. *Am J Physiol Heart Circ Physiol*. 2008;295(6):H2364-2372.
 43. Chandrasekera PC, McIntosh VJ, Cao FX, Lasley RD. Differential effects of adenosine A2a and A2b receptors on cardiac contractility. *Am J Physiol Heart Circ Physiol*. 2010;299(6):H2082-2089.
 44. Kilpatrick EL, Narayan P, Mentzer RM Jr, Lasley RD. Cardiac myocyte adenosine A2a receptor activation fails to alter cAMP or contractility: role of receptor localization. *Am J Physiol Heart Circ Physiol*. 2002;282(3):H1035-1040.
 45. Xu H, Stein B, Liang B. Characterization of a stimulatory adenosine A2a receptor in adult rat ventricular myocyte. *Am J Physiol*. 1996;270(5):H1655-1661.
 46. Grube K, Rüdibusch J, Xu Z, et al. Evidence for an intracellular localization of the adenosine A2B receptor in rat cardiomyocytes. *Basic Res Cardiol*. 2011;106(3):385-396.
 47. Xi J, McIntosh R, Shen X, et al. Adenosine A2A and A2B receptors work in concert to induce a strong protection against reperfusion injury in rat hearts. *J Mol Cell Cardiol*. 2009;47(5):684-690.
 48. Vecchio EA, White PJ, May LT. The adenosine A(2B) G protein-coupled receptor: recent advances and therapeutic implications. *Pharmacol Ther*. 2019;198:20-33.
 49. Vecchio EA, Chuo CH, Baltos JA, et al. The hybrid molecule, VCP746, is a potent adenosine A2B receptor agonist that stimulates anti-fibrotic signalling. *Biochem Pharmacol*. 2016;117:46-56.
 50. Dubey RK, Gillespie DG, Jackson EK. Adenosine inhibits collagen and protein synthesis in cardiac fibroblasts. *Hypertension*. 1998;31(4):943-948.
 51. Dubey RK, Gillespie DG, Zacharia LC, Mi Z, Jackson EK. A2B receptors mediate the antimitogenic effects of adenosine in cardiac fibroblasts. *Hypertension*. 2001;37(2):716-721.
 52. Zhang H, Zhong H, Everett TH, et al. Blockade of A2B adenosine receptor reduces left ventricular dysfunction and ventricular arrhythmias 1 week after myocardial infarction in the rat model. *Heart Rhythm*. 2014;11(1):101-109.
 53. Seo S-W, Koepfen M, Bonney S, et al. Differential tissue-specific function of Adora2b in cardioprotection. *J Immunol*. 2015;195(4):1732-1743.
 54. Grenz A, Osswald H, Eckle T, et al. The reno-vascular A2B adenosine receptor protects the kidney from ischemia. *PLoS Medicine*. 2008;5(6):e137.
 55. Hart ML, Jacobi B, Schittenhelm J, Henn M, Eltzhig HK. Cutting Edge: A2B Adenosine receptor signaling provides potent protection during intestinal ischemia/reperfusion injury. *J Immunol*. 2009;182(7):3965-3968.
 56. Cooper SL, Soave M, Jörg M, Scammells PJ, Woolard J, Hill SJ. Probe dependence of allosteric enhancers on the binding affinity of adenosine A(1) -receptor agonists at rat and human A(1) -receptors measured using NanoBRET. *Br J Pharmacol*. 2019;176(7):864-878.
 57. Szymańska E, Drabczyńska A, Karcz T, et al. Similarities and differences in affinity and binding modes of tricyclic pyrimido- and pyrazinoxanthines at human and rat adenosine receptors. *Bioorg Med Chem*. 2016;24(18):4347-4362.
 58. Kozłowska H, Schlicker E, Kozłowski M, Siedlecka U, Ludański J, Malinowska B. Ligands at beta2-, beta3-, and the low-affinity state of beta1-adrenoceptors block the alpha1-adrenoceptor-mediated constriction in human pulmonary and rat mesenteric arteries. *J Cardiovasc Pharmacol*. 2005;46(1):76-82.
 59. Mundell S, Kelly E. Adenosine receptor desensitization and trafficking. *Biochimica Et Biophysica Acta (BBA) - Biomembranes*. 2011;1808(5):1319-1328.
 60. Klaasse EC, Ijzerman AP, de Grip WJ, Beukers MW. Internalization and desensitization of adenosine receptors. *Purinergic Signal*. 2008;4(1):21-37.

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