

RESEARCH PAPER

Functional selectivity of central $G\alpha$ -subunit proteins in mediating the cardiovascular and renal excretory responses evoked by central α_2 -adrenoceptor activation *in vivo*

Correspondence

Richard D. Wainford, Department of Pharmacology and Experimental Therapeutics and the Whitaker Cardiovascular Institute, Boston University School of Medicine, 72 East Concord St, Boston, MA 02118, USA. E-mail: rwainf@bu.edu

Keywords

Brain $G\alpha$ -subunit proteins; GPCR; CNS; cardiovascular function; renal excretory function; α_2 -adrenoceptor; guanabenz

Received

22 December 2010

Revised

3 June 2011

Accepted

24 August 2011

RD Wainford^{1,2} and DR Kapusta^{1,2,3}

¹Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA, USA, ²Cardiovascular Center of Excellence, Louisiana State University Health Sciences Center, New Orleans, LA, USA, and ³Anesthesiology, Louisiana State University Health Sciences Center, New Orleans, LA, USA

BACKGROUND AND PURPOSE

Activation of brain α_2 -adrenoceptors in conscious rodents decreases heart rate (HR) and mean arterial blood pressure (MAP) and increases urine output and urinary sodium excretion. *In vitro*, α_2 -adrenoceptor stimulation activates $G\alpha_{i(1-3)}$, $G\alpha_o$ and $G\alpha_s$ -subunit protein-gated signal transduction pathways. Here we have investigated whether these same $G\alpha$ -subunit protein-gated pathways mediate the cardiovascular and renal excretory responses to central α_2 -adrenoceptor activation in conscious Sprague-Dawley rats.

EXPERIMENTAL APPROACH

Rats were pre-treated by intracerebroventricular injection (i.c.v.) with an oligodeoxynucleotide (ODN) targeted to a $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_s$ or a scrambled (SCR) ODN sequence (25 μ g, 24 h). On the day of study, the α_2 -adrenoceptor agonist guanabenz (50 μ g) or saline vehicle, was injected i.c.v. into ODN-pre-treated conscious rats. MAP and HR were recorded, and urine was collected for 150 min.

KEY RESULTS

In vehicle- and SCR ODN-pre-treated rats, i.c.v. guanabenz decreased MAP and HR, and produced marked diuretic and natriuretic responses. Selective ODN-mediated down-regulation of brain $G\alpha_{i2}$ -subunit proteins abolished the central guanabenz-induced hypotension and natriuresis. In contrast, following selective $G\alpha_s$ down-regulation, the characteristic hypotensive response to i.c.v. guanabenz was converted to an immediate increase in MAP. The bradycardic and diuretic responses to i.c.v. guanabenz were not blocked by pre-treatment with any ODN.

CONCLUSIONS AND IMPLICATIONS

There was functional selectivity of $G\alpha_{i2}$ and $G\alpha_s$ subunit protein-gated signal transduction pathways in mediating the hypotensive and natriuretic, but not bradycardic or diuretic, responses evoked by central α_2 -adrenoceptor activation *in vivo*.

Abbreviations

AVP, arginine vasopressin; BC, brain cortex; bpm, beats per minute; HR, heart rate; i.c.v., intracerebroventricular; MAP, mean arterial blood pressure; N/OFQ, nociceptin/orphanin FQ; NRG, nucleus reticularis gigantocellularis; ODN, oligodeoxynucleotide; PTX, *Pertussis* toxin; PVN, paraventricular nucleus; SCR, scrambled; UNaV, urinary sodium excretion; V, urine flow rate; VLM, ventrolateral medulla

Introduction

Multiple GPCR systems located throughout the CNS modulate systemic cardiovascular and renal excretory function through complex downstream signalling pathways. However, the specific role(s) that brain $G\alpha$ -subunit proteins (e.g. $G\alpha_{i/o}$, $G\alpha_s$) play in producing these central GPCR-mediated physiological responses *in vivo* remains essentially unknown.

The α_2 -adrenoceptor, a seven-transmembrane GPCR (Eason and Liggett, 1995; Nasman *et al.*, 2001; receptor nomenclature follows Alexander *et al.*, 2011), is highly expressed in brain regions involved in the central control of cardiovascular function and fluid and electrolyte homeostasis (Ruffolo *et al.*, 1991). When activated, central α_2 -adrenoceptors decrease heart rate (HR), mean arterial blood pressure (MAP) and central sympathetic outflow to the kidneys (Grisk and DiBona, 1998; Huang and Leenen, 1998). Concurrent with these depressor responses, stimulation of central α_2 -adrenoceptors also increases urine output and urinary sodium excretion (UNaV) (Gellai and Edwards, 1988; Menegaz *et al.*, 2001). The physiological importance that central α_2 -adrenoceptor systems play in the regulation of systemic cardiovascular function is highlighted by the therapeutic use of centrally acting α_2 -agonists for the treatment of hypertension (Degoute, 2007).

Following α_2 -adrenoceptor stimulation, intracellular GTP-binding regulatory protein coupling occurs, which triggers activation of multiple downstream signal transduction pathways. Immediately post-ligand binding, α_2 -adrenoceptors can signal via downstream $G\alpha_{i(1-3)}$, $G\alpha_o$ and $G\alpha_s$ subunit protein-gated pathways as shown in different *in vitro* model systems (Remaury *et al.*, 1993; Eason and Liggett, 1995). Through these $G\alpha$ -subunit protein pathways, activation of α_2 -adrenoceptors can lead to a range of cellular responses, including modulation of adenylate cyclase activity (inhibition via $G\alpha_{i/o}$ proteins vs. stimulation via $G\alpha_s$ proteins), inhibition of calcium channels, stimulation of potassium channels and mitogen-activated kinases (ERK1/2), all of which can modulate the activity of the CNS (Hein, 2006).

As shown in cell culture systems, the $G\alpha$ -subunit selectivity and/or protein availability may play a critical role in determining the intracellular signalling response to GPCR activation following ligand binding (Nasman *et al.*, 2001). Extending this further, in investigations performed in conscious rats, we demonstrated that the cardiovascular depressor (hypotension and bradycardia), but not diuretic, responses produced by activation of a central GPCR [the nociceptin/orphanin FQ (N/OFFQ) peptide receptor] were completely abolished in rats that had been pre-treated (48 h) centrally with *Pertussis* toxin (PTX). These findings highlight the novel functionally selective central $G\alpha$ -subunit protein-mediated control of cardiovascular versus renal excretory function *in vivo* (Wainford *et al.*, 2008). However, as PTX, which was used in these studies, is an exotoxin that catalyses the ADP ribosylation of all $G\alpha_{i/o}$ subunit proteins, the specific $G\alpha_{i(1-3)}$ or $G\alpha_o$ subunit(s) involved in mediating central GPCR-evoked cardiovascular depressor responses were not elucidated. At present, it remains unknown as to which specific $G\alpha_{i/o}$ -protein subtype(s) is involved in producing the bradycardic and hypotensive responses to the activation of

central GPCRs involved in the regulation of systemic cardiovascular haemodynamics (e.g. α_2 -adrenoceptors) *in vivo*.

Therefore, the aim of this study was to investigate the role(s) that individual brain $G\alpha_{i(1-3)}$, $G\alpha_o$ and $G\alpha_s$ subunit proteins play in the systemic cardiovascular depressor versus renal excretory responses to central α_2 -adrenoceptor activation in conscious Sprague-Dawley rats. Groups of rats were pre-treated (24 h) by intracerebroventricular (i.c.v.) injection with an oligodeoxynucleotide (ODN) sequence targeted to $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_s$ or a scrambled (SCR) ODN sequence to acutely and selectively down-regulate the respective target protein throughout the CNS (Wainford *et al.*, 2008; Wainford and Kapusta, 2010). Post-ODN treatment (24 h), we examined the cardiovascular and renal excretory responses evoked by central α_2 -adrenoceptor activation in response to i.c.v. guanabenz in conscious instrumented rats. The findings of these studies provide new insight into the intracellular mechanism of action of brain α_2 -adrenoceptors *in vivo* and demonstrate that brain $G\alpha_{i2}$ and $G\alpha_s$ subunit protein-gated signalling pathways have critical functionally selective roles in producing the hypotensive and natriuretic, but not bradycardic or diuretic, responses elicited by activation of central α_2 -adrenoceptors *in vivo*.

Methods

Animals

All animal care and experimental procedures were in accordance with National Institutes of Health and Louisiana State University Health Sciences Center Institutional Animal Care and Use Committee guidelines for the Care and Use of Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA), 275–300 g, were housed individually under a 12 h light/dark cycle. Rats were fed standard rodent diet and allowed tap water *ad libitum* and were randomly assigned to experimental treatment groups.

Measurement of MAP, HR and renal excretory function

A stainless steel cannula was stereotactically implanted into the right lateral cerebral ventricle of rats anaesthetized with ketamine (40 mg/kg, i.m.) in combination with xylazine (5 mg/kg, i.m.) 5–7 days before experimentation as previously described (Wainford *et al.*, 2008; Wainford and Kapusta, 2009; 2010). On the day of study, rats were anaesthetized with sodium methohexital (75 mg/kg i.p. and supplemented with 10 mg/kg given i.v., as needed) and instrumented with catheters in the left femoral artery, left femoral vein and bladder as described previously (Wainford *et al.*, 2008; Wainford and Kapusta, 2009; 2010). Following surgical preparation, rats were placed in a rat holder and an i.v. infusion of isotonic saline (55 μ L·min⁻¹) was started and continued for the duration of the experiment. The experimental protocol commenced after the animal regained full consciousness and systemic cardiovascular (HR and MAP) and renal excretory functions (urine output and UNaV) stabilized (4–6 h). MAP and HR were continuously recorded from the arterial cannula, which was connected to an external pressure transducer (P23XL; Viggo Spectramed, Oxnard, CA, USA); the

cardiovascular data were collected using computer-driven BIOPAC data acquisition software (MP100 and AcqKnowledge 3.8.2, BIOPAC Systems, Inc., Goleta, CA, USA). Urine was collected during a 20 min control period. Following this, guanabenz (50 μ g in 5 μ L) (Huang and Leenen, 1994; 1998) or saline vehicle (5 μ L) was injected i.c.v. ($n = 6$ per group), urine was collected during consecutive 10 min experimental periods for 150 min. Urine volume was determined gravimetrically. Urine sodium concentration was measured by flame photometry (model 943; Instrumentation Laboratories, Lexington, MA, USA) and expressed as UNaV.

G α -subunit protein ODN down-regulation studies

Rats ($n = 6$ per group) were pre-treated with a single i.c.v. injection of either a G α_{i1} ODN (25 μ g in 5 μ L, 24 h; 5'-AGAC CACTGCTTTGTA-3'), G α_{i2} ODN (25 μ g per 5 μ L, 24 h; 5'-CTT GTGCATCATCTTAGA-3'), G α_{i3} ODN (25 μ g per 5 μ L, 24 h; 5'-AAGTTGCGGTCGATCAT-3'), G α_o ODN (25 μ g per 5 μ L, 24 h; 5'-CGCCTTGCTCCGCTC-3'), G α_s ODN (25 μ g per 5 μ L, 24 h; 5'-TTGTGGCCCTCAGCGTG-3') or a SCR ODN (25 μ g per 5 μ L, 24 h; 5'-GGGGGAAGTAGGTCTTGG-3') (Rossi *et al.*, 1995; Standifer *et al.*, 1996; Hadjimarkou *et al.*, 2002). Following a National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST) search of the *Rattus norvegicus* RefSeq protein database it was confirmed that (i) there was no similarity between the SCR ODN sequence and any rat protein gene sequence and (ii) that the targeted G α -subunit ODN sequences administered in these studies were specific for their respective G α -subunit protein sequences. On the day of the experiment, 24 h after i.c.v. ODN pre-treatment injections, all animals were instrumented for measurement of cardiovascular and renal parameters and subsequently, during the experimental protocol, received a single i.c.v. injection of guanabenz (50 μ g in 5 μ L) or isotonic saline vehicle (5 μ L) (Huang and Leenen, 1994; 1998). In a subset of studies, animals received an i.c.v. injection of the α_2 -adrenoceptor antagonist yohimbine (5.9 μ g; 15 nmol) (Kapusta *et al.*, 2002) 10 min before the injection of guanabenz.

G-protein immunoblotting

Twenty-four hours following a single i.c.v. administration of saline vehicle (5 μ L) or an ODN sequence (25 μ g in 5 μ L) and completion of the acute cardiovascular and renal protocol described above, animals were then killed by decapitation; whole brains were removed and frozen at -80°C ($n = 6$ /group). Frontal brain cortex (BC), hypothalamic paraventricular nucleus (PVN) and ventrolateral medulla (VLM) samples were extracted from frozen brains cut on a cryostat using a brain punch tool (Stoelting, IL, USA). BC and PVN samples were taken using a punch diameter of 1.00 mm, VLM samples were taken using a punch diameter of 0.76 mm and were stored at -80°C . The location of the PVN and VLM was determined using visual landmarks (Paxinos and Watson, 1998; Wainford and Kapusta, 2009; 2010), and by identification of neuron populations in sections examined under a light microscope. Tissue lysates were prepared from frozen brain tissues, and protein levels were quantified. Lysates were resolved on SDS-PAGE gels and transferred to nitrocellulose membrane (GE Healthcare, Piscataway, NJ, USA). G $\alpha_{i(1-3)}$, G α_o

and G α_s levels were determined using antibodies purchased from Santa Cruz Biotechnologies (Santa Cruz, CA, USA), directed against G α_{i1} (1:100, sc-391) (Olianas *et al.*, 2007), G α_{i2} (1:200, sc-13534), G α_{i3} (1:1000, sc-262) (Bensimon *et al.*, 2004), G α_o (1:200, sc-382) and G α_s (1:1000, sc-823) (Miggin *et al.*, 2003); protein levels were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (anti-GAPDH 1:1000, ab-9483, Abcam, MA, USA) (Wainford *et al.*, 2008; Wainford and Kapusta, 2009; 2010). Chemiluminescent immunoreactive bands were detected by horseradish peroxidase-conjugated secondary antibody; data were imaged and quantified using Bio-Rad Quantity One image analysis software (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

All data are expressed as mean $\pm$ SEM. The magnitude of the changes in cardiovascular and renal excretory parameters at different time points after i.c.v. injection of guanabenz were compared with respective group control values by a one-way repeated measures ANOVA with subsequent Dunnett's test. Differences occurring between treatment groups (e.g. i.c.v. SCR ODN vs. G α_{i2} ODN) were assessed by a two-way repeated measure ANOVA with pre-treatment group (e.g. saline vehicle) being one fixed effect and time the other, with the interaction included. The time (minutes) was then the repeated factor. *Post hoc* analysis was performed using Bonferroni's test. Where appropriate, a Student's *t*-test was also used to compare means between two groups. In each case, statistical significance was defined as $P < 0.05$.

Materials

Guanabenz and yohimbine were supplied by Sigma Aldrich, St. Louis, MO; ketamine by Vedco Inc., St. Joseph, MO; xylazine by Butler, Columbus, OH; methohexital by JHP Pharmaceuticals, LLC, Rochester, MI.

Results

SCR ODN pre-treatment does not alter the cardiorenal responses to i.c.v. guanabenz

In conscious Sprague-Dawley rats pre-treated with isotonic saline vehicle (5 μ L, 24-h), i.c.v. guanabenz (50 μ g) produced characteristic concurrent reductions in HR and MAP followed by a delayed onset increase in urine flow rate and UNaV (Figure 1). Peak bradycardia and hypotension were observed 20 min and 30 min following i.c.v. guanabenz administration respectively. HR and MAP remained significantly depressed for 40 min, returning to baseline control levels by 60 min after guanabenz injection. The maximal diuretic response was observed 80–90 min after guanabenz injection, with urine flow rate returning to pre-drug control levels by the end of the experimental period (150 min). No change in UNaV was detected until 80 min after guanabenz administration; from which time point thereafter, a significant natriuretic response was observed for the duration of the experimental protocol. Central administration of the α_2 -adrenoceptor antagonist yohimbine (5.9 μ g; 15 nmol) 10 min before the i.c.v. injection of guanabenz (50 μ g) completely blocked the cardiovascular and renal excretory responses to i.c.v. guanabenz (Figure 1).

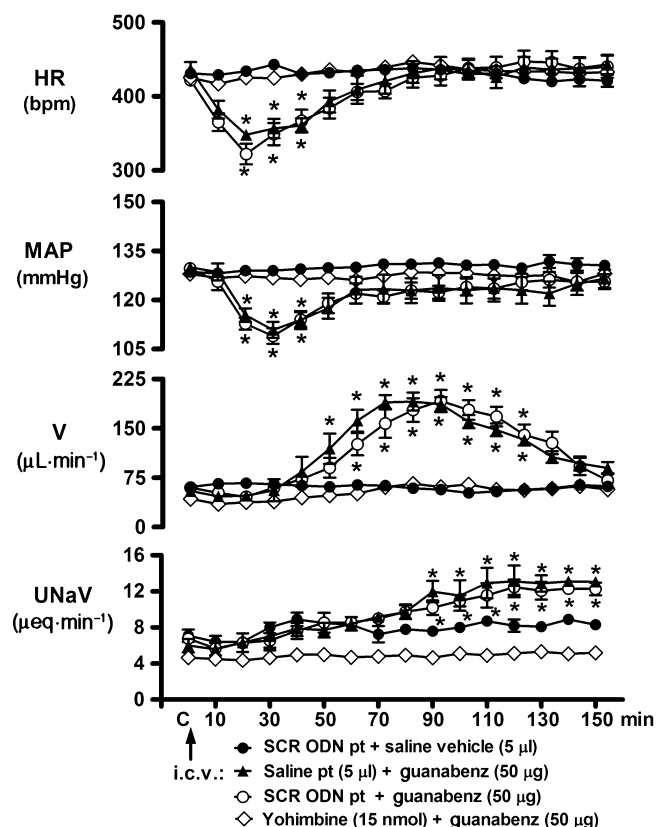


Figure 1

Effect of i.c.v. SCR ODN pre-treatment (pt) on the cardiovascular and renal excretory responses to central guanabenz administration in conscious male Sprague-Dawley rats. The values are means $\pm$ SEM and illustrate the cardiovascular and renal effects of i.c.v. guanabenz (50 μ g) in six conscious rats per group that were pre-treated for 24 h with i.c.v. SCR ODN (25 μ g in 5 μ L) or isotonic saline vehicle (5 μ L) or received an i.c.v. pre-treatment of the α_2 -adrenoceptor antagonist yohimbine (15 nmol) 10 min prior to the administration of i.c.v. guanabenz. V, urine flow rate. * P < 0.05, compared with respective group control value (designated C); one-way repeated measures ANOVA.

Pre-treatment with SCR ODN (25 μ g in 5 μ L, i.c.v., for 24 h) did not alter baseline control cardiovascular or renal excretory parameters (shown as C; Figure 1) or animal body weight (SCR ODN, $\Delta +3 \pm 2$ g). As illustrated (Figure 1; open circles), SCR ODN pre-treatment did not alter either the duration or magnitude of the cardiovascular depressor or renal excretory responses to the central administration of guanabenz. Further, i.c.v. administration of isotonic saline (5 μ L) did not alter any cardiovascular or renal parameter under investigation over the duration of the experimental protocol in SCR ODN-pre-treated animals (Figure 1).

Selective central ODN-mediated down-regulation of brain α -subunit protein expression

Immunoblotting studies revealed regional differences in the endogenous expression of α -subunits within the brain of

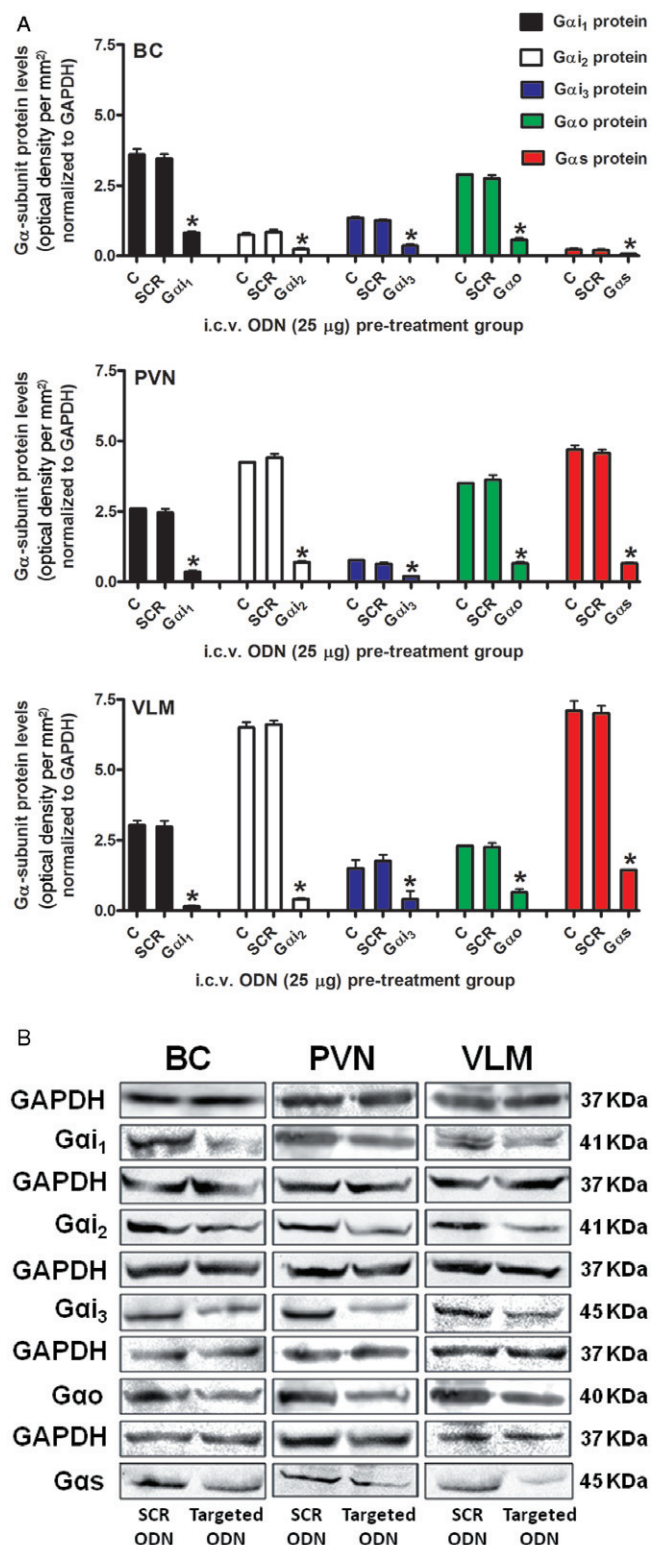
male Sprague-Dawley rats. α_{10} protein was expressed at equivalent levels in the BC and PVN, with lower levels detected in the VLM (Figure 2A). The expression of α_{41} protein was greatest in BC with lower but comparable levels present in PVN and VLM tissue. α_{43} protein was present at a low level in all three brain regions examined. BC tissue had low expression levels of both α_{42} and α_5 subunit proteins; in contrast both the PVN and VLM exhibited high expression levels of these subunit proteins. SCR ODN pre-treatment did not alter brain α -subunit protein expression levels in any tissue examined (Figure 2A and B).

As observed for SCR ODN pre-treatment, i.c.v. α_{41} , α_{42} , α_{43} , α_{10} , or α_5 ODN pre-treatment (each 25 μ g in 5 μ L for 24 h) did not significantly alter animal body weight (α_{41} ODN, $\Delta -3 \pm 1$ g; α_{42} ODN, $\Delta +4 \pm 3$ g; α_{43} ODN, $\Delta -3 \pm 2$ g; α_{10} ODN, $\Delta -5 \pm 3$ g; α_5 ODN, $\Delta +2 \pm 1$ g) or baseline control levels for cardiovascular and renal excretory function (corresponding group values denoted 'C' in Figures 5 and 6, data not shown). Following 24 h central ODN pre-treatment, α_{41} , α_{42} , α_{43} , α_{10} and α_5 ODN sequences reduced respective target α protein expression levels in the BC, PVN and VLM (Figure 2A and B). α subunit protein levels were reduced significantly, compared with both naïve animals and animals pre-treated i.c.v. with a non-specific control SCR ODN sequence, by α -subunit protein targeted ODN's in a sequence specific manner. ODN-mediated protein down-regulation resulted in at least 70% reduction in the respective target protein in the BC compared with naïve rats or SCR ODN-pre-treated animals. Down-regulation of target proteins was greater in PVN and VLM tissue with a reduction in protein expression of at least 80 and 85% in PVN and VLM tissue respectively. These findings demonstrate successful ODN dispersal throughout the brain and efficacy in reducing the target protein following a single i.c.v. injection at a dose of 25 μ g following a pre-treatment time period of 24 h.

Additional immunoblotting studies were performed to confirm the *in vivo* selectivity of α -subunit targeted ODN sequences (Figure 3A and B). In these studies, brain tissue samples from all ODN-treated groups for which physiological data are presented in Figures 4–6 were examined for the expression of all non-targeted α -subunit proteins under investigation (i.e. α_{41} , α_{42} , α_{43} and α_{10} expression in a α_5 ODN-pre-treated group). As illustrated (Figure 3A and B), the central pre-treatment (25 μ g, 24 h) of male Sprague-Dawley rats with a targeted α -subunit ODN sequence resulted in highly significant and selective target protein down-regulation.

Functionally selective effects of central α -subunit proteins in the cardiovascular depressor responses to central guanabenz

Following i.c.v. guanabenz injection, the peak changes in HR and MAP were typically observed 20 min and 30 min post injection, respectively, and were completely blocked by i.c.v. pre-treatment with the α_2 -adrenoceptor antagonist yohimbine (Figure 1, time course responses; Figure 4A and B, peak magnitudes). Pre-treatment of animals with a SCR ODN sequence did not alter the magnitude or timing of peak guanabenz-induced bradycardia or hypotension (Figure 4A and B). I.c.v. pre-treatment of rats with an ODN selective for α_{10} was the only ODN pre-treatment that significantly



reduced the magnitude of the central guanabenz-evoked bradycardia. I.c.v. pre-treatment with Gα_{i3} and Gα_s ODN sequences resulted in an observable, but non-statistically significant, reduction (approximately 30%) in the magnitude of the bradycardic response (Figures 4A and 6). In contrast, central Gα_{i1} and Gα_{i2} ODN pre-treatments had no effect on guanabenz-evoked bradycardia.

Figure 2

(A) Effect of central targeted Gα-subunit ODN pre-treatment on Gα-subunit protein levels normalized to GAPDH and expressed as optical density units per mm² (mean ± SEM) in the BC, hypothalamic PVN and VLM of male Sprague-Dawley rats pre-treated (24 h) with i.c.v. either saline (5 μL) or a SCR (SCR), Gα_{i1}, Gα_{i2}, Gα_{i3}, Gα_o or Gα_s ODN (25 μg in 5 μL each) (n = 6 per group), and (B) Representative immunoblots illustrating GAPDH, Gα_{i1}, Gα_{i2}, Gα_{i3}, Gα_o and Gα_s subunit protein levels in the BC, PVN and VLM from male Sprague-Dawley rats pre-treated (24 h; i.c.v.) with either a selective Gα-subunit protein-targeted ODN sequence or a SCR ODN sequence (25 μg in 5 μL each). *P < 0.05, compared with i.c.v. SCR ODN-pre-treated animal group value; unpaired Student's *t* test.

I.c.v. pre-treatment with a Gα_{i1}, Gα_{i3} or a Gα_o ODN did not alter the peak hypotensive response to the central administration of guanabenz (Figure 4B). Further, although not shown, these ODN sequences did not alter the time course of the hypotensive response observed following central guanabenz administration, (illustrated in Figure 1) in saline and vehicle pre-treated animals. In contrast to these findings, central Gα_{i2} ODN pre-treatment completely abolished the hypotensive response to i.c.v. guanabenz. However, as demonstrated in Figures 4A and 5 (time course studies), central down-regulation of Gα_{i2} subunit proteins failed to prevent central guanabenz-evoked bradycardia. In contrast, in central Gα_s ODN-pre-treated rats (Figures 4A and 6), i.c.v. guanabenz produced an immediate increase in MAP resulting in a significant hypertensive response. This was the opposite response to the characteristic hypotension produced by i.c.v. injection of guanabenz. As shown in Figure 6 (time course studies), the pressor response to i.c.v. guanabenz in Gα_s pre-treated rats was of short duration with MAP returning to control levels by 20 min after agonist administration.

Functionally selective effects of central Gα-subunit proteins in the diuretic and natriuretic responses to central guanabenz

In both saline vehicle and SCR ODN-pre-treated groups, i.c.v. guanabenz produced a profound, but with delayed onset, diuresis, which in both groups featured an identical peak increase in urine output, was of a comparable magnitude and time course between the two experimental groups (Table 1 and Figure 1). Central pre-treatment with a Gα_{i1}, Gα_{i2}, Gα_{i3}, Gα_o, or a Gα_s ODN did not alter the diuretic response to i.c.v. guanabenz. There were no significant differences observed between these experimental groups in cumulative urine output, peak change in urine output or, as illustrated for Gα_{i2} and Gα_s pre-treatments (data from other groups not shown), the duration of the diuretic response (Table 1, Figures 5 and 6).

Following central saline vehicle or SCR ODN pre-treatment, i.c.v. guanabenz evoked a significant and prolonged increase in UNaV (Figure 1, Table 1). Prior down-regulation of central Gα_{i1}, Gα_{i3} or Gα_o subunit proteins did not alter the magnitude (Table 1) or time course (data not shown) of the natriuretic response evoked by i.c.v. guanabenz. The natriuretic response to central administration of guanabenz was completely abolished in rats pre-treated with

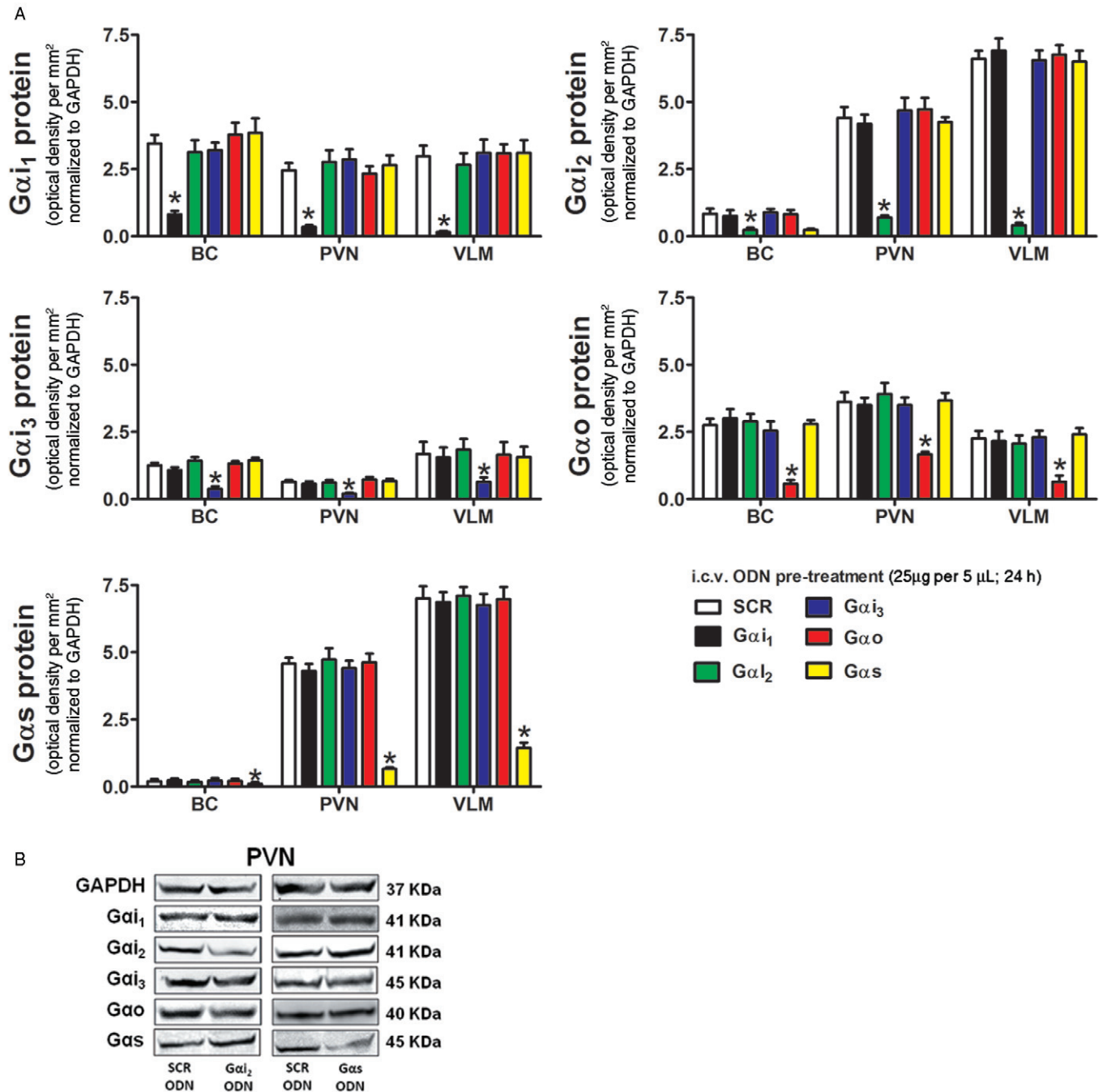


Figure 3

(A) Selectivity of central $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$ and $G\alpha_s$ targeted ODN pre-treatment on $G\alpha$ -subunit protein levels normalized to GAPDH and expressed as optical density units per mm^2 (mean $\pm$ SEM) in the BC, hypothalamic PVN and VLM of male Sprague-Dawley rats pre-treated (24 h; i.c.v.) with a SCR, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$ or $G\alpha_s$ ODN (25 μ g in 5 μ L each) ($n = 6$ per group) * $P < 0.05$, compared with i.c.v. SCR ODN-pre-treated animal group value; one-way repeated measures ANOVA and (B) representative immunoblots illustrating GAPDH, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$ and $G\alpha_s$ subunit protein levels in the PVN from male Sprague-Dawley rats pre-treated (24 h; i.c.v.) with a $G\alpha_{i2}$ or $G\alpha_s$ ODN sequence.

a $G\alpha_{i2}$ ODN (Table 1). Although the peak natriuretic response to i.c.v. guanabenz was not altered in $G\alpha_s$ ODN-pre-treated rats (Table 1), the time course of the natriuresis was significantly altered in this treatment group (Figure 6). More specifically, in $G\alpha_s$ ODN-pre-treated rats the natriuresis produced by i.c.v. guanabenz was observed immediately following drug

administration with the peak increase in UNaV observed at 30 min after drug administration (Figure 6). In this group, UNaV returned to pre-drug control levels 50 min after guanabenz injection and remained at basal levels for the remainder of the 150 min experimental protocol. As previously noted, this pattern of natriuresis is in contrast to the renal

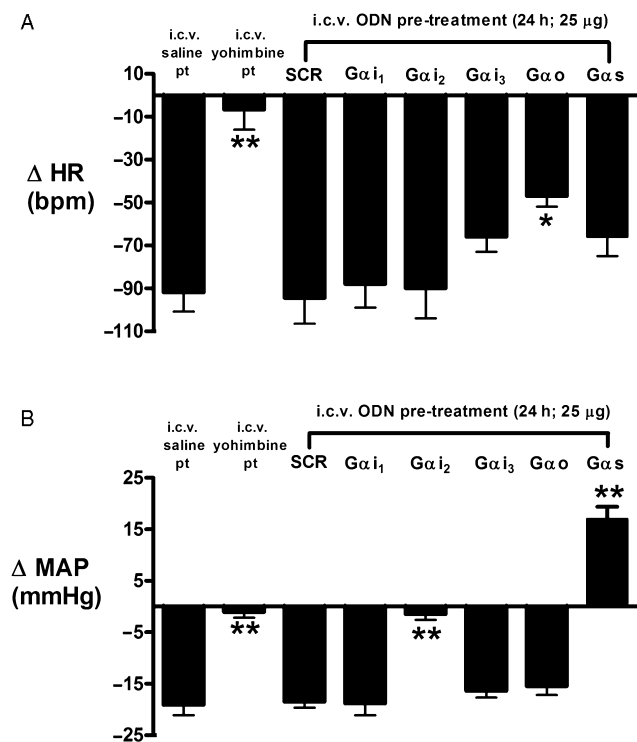


Figure 4

(A) Peak HR depressor response in bpm and (B) peak MAP depressor response in mmHg produced by i.c.v. guanabenz (50 µg in 5 µL) in conscious male Sprague-Dawley rats pre-treated i.c.v. with either saline (24 h; 5 µL), yohimbine (10 min; 5.9 µg; 15 nmol) or a SCR, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$ or $G\alpha_s$ ODN (24 h; 25 µg in 5 µL each) (n = 6 per group). The values are the mean $\pm$ SEM. * P < 0.05, compared with i.c.v. SCR ODN-pre-treated animal group value. ** P < 0.01, compared with i.c.v. SCR ODN-pre-treated animal group value; one-way ANOVA. Note that these data were obtained in the same animals for which protein expression data is presented in Figure 2.

excretory response produced by central guanabenz in SCR ODN-pre-treated rats, in which the natriuresis was delayed in onset with a peak response at 120 min after agonist administration (Figures 1 and 6).

Discussion

Through the central administration of selective $G\alpha$ -subunit protein-targeted ODNs, we have demonstrated that brain $G\alpha_{i2}$ and $G\alpha_s$ subunit protein-gated signalling pathways are critical for mediating the hypotensive and natriuretic, but not bradycardic and diuretic, responses produced by the central administration of the α_2 -adrenoceptor agonist guanabenz in conscious rats. These observations provide compelling *in vivo* support for the pharmacological paradigm of functional selectivity (Patel *et al.*, 2010). These findings also establish that *in vivo*, the physiological responses produced by the activation of a CNS GPCR, in this case, the α_2 -adrenoceptor, is greatly influenced by the availability and/or brain protein expression levels of individual down-

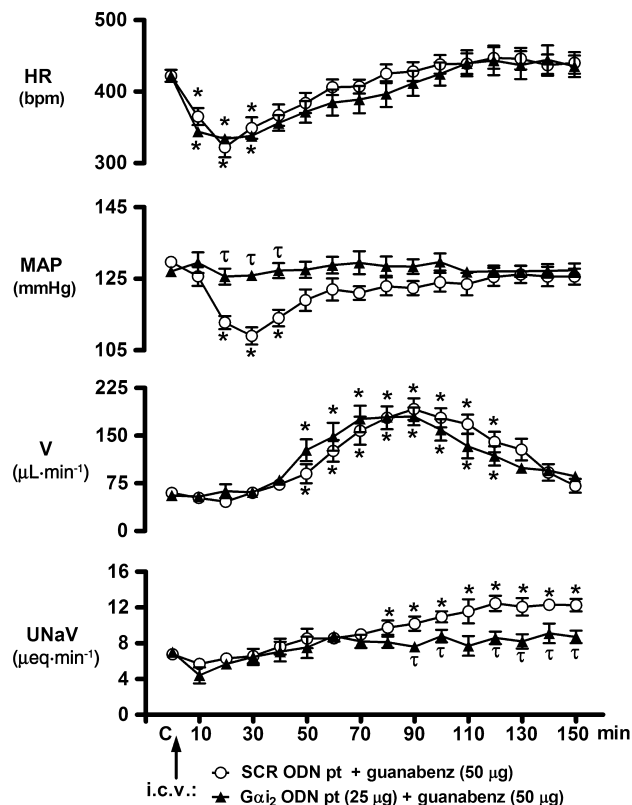


Figure 5

Effect of i.c.v. $G\alpha_{i2}$ ODN pre-treatment on the cardiovascular and renal excretory responses to central guanabenz administration in conscious male Sprague-Dawley rats. The values are means $\pm$ SEM and illustrate the cardiovascular and renal effects of i.c.v. guanabenz (50 µg) in six conscious rats per group that were pre-treated (24 h) with i.c.v. SCR ODN (25 µg per 5 µL) or a $G\alpha_{i2}$ ODN (25 µg in 5 µL). Data for SCR ODN pre-treatment (pt) are transposed from Figure 1 for comparison and clarity. Abbreviations as in Figure 1. * P < 0.05, compared with respective group control value (designated C); one-way repeated measures ANOVA. $^{\dagger}P$ < 0.05, compared with SCR ODN pt group value at respective time point; two-way repeated measures ANOVA.

stream $G\alpha$ -subunit proteins. Thus, following a single ligand-receptor interaction, multiple downstream signalling pathways are differentially activated to evoke multiple integrated cardiovascular versus renal excretory responses.

In vivo, α_2 -adrenoceptor agonists inhibit sympathetic outflow to many organs to facilitate the reported cardiovascular depressor and renal excretory responses produced by these GPCR ligands. Particularly well established is the relationship between the decrease in renal sympathetic nerve activity and the natriuretic response observed following central administration of different α_2 -adrenoceptor agonists (Grisk and DiBona, 1998; Huang and Leenen, 1998). Our observation that both the hypotensive and natriuretic responses to i.c.v. guanabenz are abolished in animals pre-treated centrally with $G\alpha_{i2}$ ODN is of high physiological importance and highlights a key role for $G\alpha_{i2}$ protein pathways in regulating MAP and UNaV post CNS α_2 -adrenoceptor activation. Further, this finding validates existing *in vitro* data

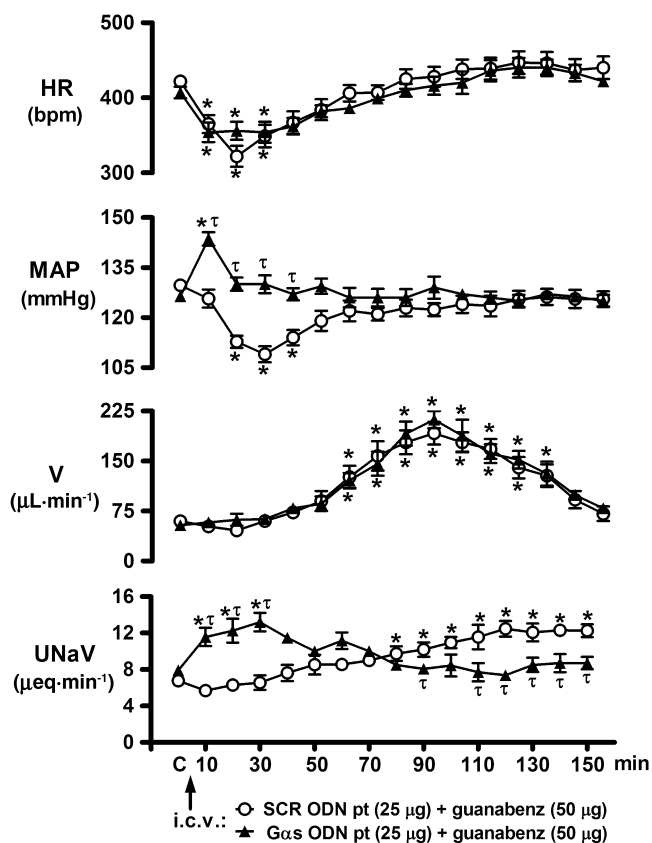


Figure 6

Effect of i.c.v. $G\alpha_s$ ODN pre-treatment on the cardiovascular and renal excretory responses to central guanabenz administration in conscious male Sprague-Dawley rats. The values are means $\pm$ SEM and illustrate the cardiovascular and renal effects of i.c.v. guanabenz (50 μ g) in six conscious rats per group that were pre-treated (24 h) with i.c.v. SCR ODN (25 μ g in 5 μ L) or a $G\alpha_s$ ODN (25 μ g in 5 μ L). Data for SCR ODN pre-treatment (pt) are transposed from Figure 1 for comparison and clarity. Abbreviations as in Figure 1. * P < 0.05, compared with respective group control value (designated C); one-way repeated measures ANOVA. # P < 0.05, compared with SCR ODN pt group value at respective time point; two-way repeated measures ANOVA.

(Remaury *et al.*, 1993; Eason and Liggett, 1995) and confirms a role of $G\alpha_{i2}$ -subunit proteins in mediating α_2 -adrenoceptor-evoked responses. The selectivity of this response profile to $G\alpha_{i2}$ and not $G\alpha_{i1}$, $G\alpha_{i3}$ or $G\alpha_o$ -subunit proteins also suggests that this response is not conserved across $G\alpha_{i/o}$ subunit proteins. This selective response profile compares favourably with a previous *in vivo* study conducted in mice in which the antinociceptive effects of three different α_2 -adrenoceptor agonists administered i.c.v. were selectively mediated by $G\alpha_{i3}$ -subunit proteins (Raffa *et al.*, 1996). Although not examined in these studies, we speculate that prior down-regulation of central $G\alpha_{i2}$ proteins in rats caused a failure of i.c.v. guanabenz to inhibit renal sympathetic nerve activity, the action of which is likely to be responsible for preventing the natriuretic response to this ligand. However, as shown by the concurrent blockade of the hypotensive response to i.c.v. guanabenz, it appears that down-regulation of central $G\alpha_{i2}$ proteins may

have also prevented the reduction in sympathetic nerve traffic to additional vascular beds (e.g. splanchnic) involved in the maintenance of systemic arterial MAP. In contrast, these data suggest that suppression of central sympathetic outflow to the heart is not impaired as shown by the classical bradycardic response that was still produced by i.c.v. guanabenz in $G\alpha_{i2}$ -ODN-treated animals. In these studies, the bradycardia to i.c.v. guanabenz was significantly attenuated by $G\alpha_o$ subunit protein down-regulation, data that suggest a predominant role of $G\alpha_o$ -subunits in the guanabenz-evoked signalling pathways that mediate bradycardia. Taken in conjunction with our previous finding that the bradycardia evoked by i.c.v. administration of the opioid-like neuropeptide N/OFQ was abolished by pre-treatment with PTX (Wainford *et al.*, 2008), which inhibits the activity of all $G\alpha_{i/o}$ subunit proteins, these data suggest that there is potential functional redundancy across $G\alpha$ -subunit protein-gated pathways to elicit bradycardia following guanabenz administration. Alternatively, guanabenz evoked bradycardia may be mediated via a mechanism independent of GPCR $G\alpha$ -subunit signalling (e.g. $\beta\gamma$ or β -arrestin).

As discussed above, selective down-regulation of brain $G\alpha_{i2}$ proteins completely prevented the hypotensive response to central α_2 -adrenoceptor stimulation in conscious rats. In the present investigation, we also observed the highly unexpected finding that the selective ODN-mediated down-regulation of brain $G\alpha_s$ -subunit proteins converted the classical hypotensive response to central α_2 -adrenoceptor activation into an immediate and profound hypertensive response. With regards to the immediate natriuresis observed in $G\alpha_s$ ODN-pre-treated animals, we believe that this physiological response was a result of the rapid elevation in MAP (i.e. a pressure natriuresis), which occurred immediately following i.c.v. guanabenz. Collectively, these findings provide strong *in vivo* evidence that $G\alpha$ -subunit protein availability can significantly modulate the physiologically important cardiovascular and renal excretory responses to ligand binding, as has been predicted by *in vitro* studies and by mathematical modelling (Nasman *et al.*, 2001; Hein, 2006).

In the present studies the cellular mechanism(s) by which prior down-regulation of brain $G\alpha_{i2}$ or $G\alpha_s$ proteins altered the MAP responses to central guanabenz was not determined. However, *in vitro* experiments have demonstrated that the predominant cellular action of α_2 -adrenoceptors, which is mediated principally via $G\alpha_{i2}$ -subunit protein signal transduction, is the inhibition of adenylate cyclase activity (Remaury *et al.*, 1993; Nasman *et al.*, 2001; Hein, 2006). Therefore, we hypothesize that following stimulation of brain α_2 -adrenoceptors, activation of a downstream $G\alpha_{i2}$ -subunit protein-gated pathway triggers the suppression of central sympathetic outflow by a signalling pathway involving, at least in part, inhibition of adenylate cyclase activity. Based on this premise, down-regulation of $G\alpha_{i2}$ proteins in the brain may abolish the central sympathoinhibitory and thus the hypotensive and natriuretic responses to central guanabenz by preventing the α_2 -adrenoceptor-coupled inhibition of cAMP. A functional coupling to $G\alpha_s$ has also been reported for α_2 -adrenoceptors, which is apparent at high agonist concentration or after inhibition of $G\alpha_i$ (Eason and Liggett, 1995; Wade *et al.*, 1999). While this sequence of events is possible and has been suggested to participate in mediating the phe-

Table 1

Effects of pre-treatment (i.c.v.) with ODNs (for $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_s$ or SCR) on the diuretic and natriuretic responses to i.c.v. guanabenz in conscious Sprague-Dawley rats

i.c.v. ODN pre-treatment (24 h; 25 μ g)	Cumulative urine output (μ L per 150 min)	Peak Δ urine output (μ L $\cdot$ min $^{-1}$)	Peak Δ UNaV (μ eq $\cdot$ min $^{-1}$)
Saline vehicle	10 569 $\pm$ 619	136 $\pm$ 9	6.2 $\pm$ 0.8
Scrambled (SCR)	9 998 $\pm$ 496	132 $\pm$ 11	5.9 $\pm$ 0.6
$G\alpha_{i1}$	10 246 $\pm$ 513	153 $\pm$ 16	6.1 $\pm$ 0.8
$G\alpha_{i2}$	9 933 $\pm$ 361	125 $\pm$ 11	0.6 $\pm$ 0.3**
$G\alpha_{i3}$	10 960 $\pm$ 691	143 $\pm$ 16	6.2 $\pm$ 0.9
$G\alpha_o$	10 171 $\pm$ 514	127 $\pm$ 10	6.2 $\pm$ 0.5
$G\alpha_s$	10 654 $\pm$ 461	150 $\pm$ 13	5.8 $\pm$ 0.6

The values are means $\pm$ SEM and illustrate the diuretic (expressed as cumulative urine output in μ L per 150 min and peak change in urine output in μ L $\cdot$ min $^{-1}$) and natriuretic [expressed as peak change in urinary sodium excretion (UNaV) in μ eq $\cdot$ min $^{-1}$] effects of i.c.v. guanabenz (50 μ g) in six conscious rats per group that were pre-treated (24 h) with an ODN (25 μ g in 5 μ L) or isotonic saline vehicle (5 μ L). ** P < 0.01, compared with SCR ODN pre-treatment group value.

nomenon of adenylate cyclase supersensitivity (Watts and Neve, 2005), it remains to be explained why i.c.v. guanabenz produced an immediate and profound hypertensive response in rats that had undergone prior down-regulation of brain $G\alpha_s$ proteins. It is also possible that the observed functional selectivity of $G\alpha_{i2}$ and $G\alpha_s$ subunit proteins to mediate the hypotensive and natriuretic effects evoked by activation of brain α_2 -adrenoceptors reflects an alteration of the inhibition of Ca^{2+} channels in sympathetic neurons. As has been demonstrated *in vitro*, altered inhibition of Ca^{2+} channels in sympathetic neurones, mediated by adrenoceptors, can occur via PTX-sensitive (i.e. $G\alpha_{i/o}$ pathways) and non-PTX-sensitive (e.g. $G\alpha_s$) pathways, or via modulation of the composition of the β protein dimer associated with the α -subunit (Delmas *et al.*, 1999).

In contrast to our current findings is a recent report that whole body deletions of the genes encoding the $G\alpha_{i(1-3)}$ subunit proteins in mice did not alter the cardiovascular depressor effects (i.e. bradycardia and hypotension) evoked by peripheral administration of the α_2 -adrenoceptor agonist medetomidine (Albarran-Juarez *et al.*, 2009). While the reasons for these contrasting findings have yet to be determined, the divergence may be partially explained by differences in species of animals tested, the mode by which removal/down-regulation of specific $G\alpha$ -subunit proteins was achieved [e.g. transgenic whole body deletions of target genes vs. CNS delivery of targeted $G\alpha$ -subunit ODNs], presence/absence of anaesthesia and route of administration (i.v. bolus vs. i.c.v. injection) of different α_2 -adrenoceptor agonists (medetomidine vs. guanabenz).

Following i.c.v. injection and circulation throughout the brain, it is likely that guanabenz activates $G\alpha_{i2}$ / $G\alpha_s$ -subunit protein-gated pathways located within several brain sites involved in the regulation of cardiovascular and fluid/electrolyte homeostasis. This may include, but is not limited to, the PVN of the hypothalamus, the rostral VLM (RVLM) and the nucleus reticularis gigantocellularis (NRGC) (Chen and Chan, 1989; Menegaz *et al.*, 2001). The neurons located within these established renal and cardiovascular control

centres possess significant quantities of α_2 -adrenoceptors (Tavares *et al.*, 1996), which may potentially be coupled to different $G\alpha$ -subunit proteins as a mechanism to elicit differential physiological responses (i.e. functional selectivity). However, the current studies did not determine the locus of the reported $G\alpha_{i2}$ and $G\alpha_s$ -subunit protein interactions within the CNS that occur downstream of the α_2 -adrenoceptor. Site-specific microinjection experiments (e.g. into discrete brain sites), which are beyond the scope of the current manuscript, will be conducted in subsequent experiments to establish the CNS sites in which individual $G\alpha$ -subunit protein pathways act to produce the observed responses to central α_2 -adrenoceptor stimulation. An additional issue not addressed by the current studies is the subtype of α_2 -adrenoceptor (i.e. α_{2A} , α_{2B} or α_{2C}) that is activated by i.c.v. guanabenz administration. The α_{2A} receptor is widely distributed throughout the CNS, particularly in key cardiovascular control centres such as PVN, VLM and locus coeruleus (Scheinin *et al.*, 1994), and has an established role in modulating neurotransmitter release (Lahdesmaki *et al.*, 2004). Therefore, it is likely this subtype may play a predominant role in the observed responses. However, due to the expression of the α_{2B} receptor in the thalamus and expression of the α_{2C} -subtype, which can also modulate neurotransmitter release, in many brain sites (Scheinin *et al.*, 1994), a role of these subtypes in the observed responses cannot be excluded.

In contrast to effects on MAP and UNaV, the prior ODN-mediated down-regulation of central $G\alpha_{i/o}$ or $G\alpha_s$ -subunit proteins did not alter the diuretic responses to i.c.v. guanabenz. This observation indicates that other signal transduction pathways are involved in mediating the effects of central α_2 -adrenoceptors on the renal handling of water. Our laboratory has previously established that exogenous (Wainford *et al.*, 2008) or endogenous (Wainford and Kapusta, 2010) activation of central GPCR pathways can influence urine output via differentially altering the secretion of arginine vasopressin (AVP) through a pathway involving downstream central $G\alpha_z$ (inhibitory) and $G\alpha_q$ (stimulatory) subunit pro-

teins. A significant component of the diuretic response elicited by central α_2 -adrenoceptor stimulation is mediated by the suppression of AVP secretion (Brooks *et al.*, 1986; Cabral *et al.*, 1998). Thus, the pattern of diuresis produced by central α_2 -adrenoceptors may also involve alterations in AVP secretion mediated via $G_{\alpha_z}/G_{\alpha_q}$ -subunit protein-gated pathways.

In conclusion, these studies significantly advance our understanding of the functional selectivity of brain α -subunit protein-gated pathways in the physiological responses evoked by central α_2 -adrenoceptor activation. Using a targeted ODN approach *in vivo*, we demonstrated that the hypotensive and natriuretic responses to selective central α_2 -adrenoceptor activation are abolished by $G_{\alpha_{i2}}$ down-regulation or converted to a pressor response by G_{α_s} down-regulation. In contrast, the profound α_2 -agonist-stimulated diuresis was unaltered by prior down-regulation of individual brain $G_{\alpha_{i(1-3)}}$, G_{α_s} or G_{α_o} proteins. Collectively, these findings demonstrate that central α -subunit protein-gated pathways play a functionally selective role in producing differential cardiovascular versus renal excretory responses post-ligand binding at α_2 -adrenoceptors. In response to integrated physiological stimuli, selective activation of individual central α -subunit pathways may potentially play an important role in governing the endogenous regulation of systemic cardiovascular function and fluid and electrolyte homeostasis. Therefore, we suggest modulation of either the expression and/or activity of individual endogenous α -subunit proteins represents a target area for the future development of pharmacological therapies designed to alter systemic cardiovascular parameters (e.g. antihypertensive medications) versus renal excretory function (water diuretics, natriuretic compounds).

Acknowledgements

The research presented in this manuscript was funded to RDW by the American Heart Association Grant 09BGIA2250585 and the National Institute of Health Grant NHLBI HL 107330 to DRK by the National Institute of Health grants NIDDK DK43337, NHLBI HL 071212 and the American Heart Association grant 08552930 and to RDW/DRK by the NIH NCRR COBRE P20 RR018766 grant.

Conflicts of interest

None.

References

- Albarran-Juarez J, Gilsbach R, Piekorz RP, Pexa K, Beetz N, Schneider J *et al.* (2009). Modulation of α_2 -adrenoceptor functions by heterotrimeric Galphai protein isoforms. *J Pharmacol Exp Ther* 331: 35–44.
- Alexander SPH, Mathie A, Peters JA (2011). Guide to Receptors and Channels (GRAC), 5th Edition. *Br J Pharmacol* 164 (Suppl. 1): S1–S324.
- Bensimon M, Chang AI, de Bold ML, Ponce A, Carreras D, De Bold AJ (2004). Participation of G proteins in natriuretic peptide hormone secretion from heart atria. *Endocrinology* 145: 5313–5321.
- Brooks DP, Share L, Crofton JT (1986). Central adrenergic control of vasopressin release. *Neuroendocrinology* 42: 416–420.
- Cabral AD, Kapusta DR, Kenigs VA, Varner KJ (1998). Central α_2 -receptor mechanisms contribute to enhanced renal responses during ketamine-xylazine anesthesia. *Am J Physiol* 275: R1867–R1874.
- Chen CH, Chan SH (1989). Involvement of postsynaptic α_2 -adrenoceptors and guanine nucleotide-binding protein in guanabenz-induced cardiovascular suppressant effects in the rat. *Neurosci Lett* 105: 183–188.
- Degoute CS (2007). Controlled hypotension: a guide to drug choice. *Drugs* 67: 1053–1076.
- Delmas P, Abogadie FC, Milligan G, Buckley NJ, Brown DA (1999). $\beta\gamma$ dimmers derived from G_o and G_i proteins contribute different components of adrenergic inhibition of Ca^{2+} channels in rat sympathetic neurons. *J Physiol* 518: 23–36.
- Eason MG, Liggett SB (1995). Identification of a G_s coupling domain in the amino terminus of the third intracellular loop of the α_{2A} -adrenergic receptor. Evidence for distinct structural determinants that confer G_s versus G_i coupling. *J Biol Chem* 270: 24753–24760.
- Gellai M, Edwards RM (1988). Mechanism of α_2 -adrenoceptor agonist-induced diuresis. *Am J Physiol* 255: F317–F323.
- Grisk O, DiBona GF (1998). Influence of arterial baroreceptors and intracerebroventricular guanabenz on synchronized renal nerve activity. *Acta Physiol Scand* 163: 209–218.
- Hadjimarkou MM, Silva RM, Rossi GC, Pasternak GW, Bodnar RJ (2002). Feeding induced by food deprivation is differentially reduced by G-protein α -subunit antisense probes in rats. *Brain Res* 955: 45–54.
- Hein L (2006). Adrenoceptors and signal transduction in neurons. *Cell Tissue Res* 326: 541–551.
- Huang BS, Leenen FH (1994). Brain 'ouabain' mediates the sympathoexcitatory and hypertensive effects of high sodium intake in Dahl salt-sensitive rats. *Circ Res* 74: 586–595.
- Huang BS, Leenen FH (1998). Both brain angiotensin II and 'ouabain' contribute to sympathoexcitation and hypertension in Dahl S rats on high salt intake. *Hypertension* 32: 1028–1033.
- Kapusta DR, Dayan LA, Kenigs VA (2002). Nociceptin/orphanin FQ modulates the cardiovascular, but not renal, responses to stress in spontaneously hypertensive rats. *Clin Exp Pharmacol Physiol* 29: 254–259.
- Lahdesmaki J, Sallinen J, MacDonald E, Scheinin M (2004). α_2A -Adrenoceptors are important modulators of the effects of d-amphetamine on startle reactivity and brain monoamines. *Neuropsychopharmacology* 29: 1282–1293.
- Menegaz RG, Kapusta DR, Mauad H, Cabral AM (2001). Activation of α_2 -receptors in the rostral ventrolateral medulla evokes natriuresis by a renal nerve mechanism. *Am J Physiol* 281: R98–R107.
- Miggin SM, Lawler OA, Kinsella BT (2003). Palmitoylation of the human prostacyclin receptor. Functional implications of palmitoylation and isoprenylation. *J Biol Chem* 278: 6947–6958.

- Nasman J, Kukkonen JP, Ammoun S, Akerman KE (2001). Role of G-protein availability in differential signaling by alpha 2-adrenoceptors. *Biochem Pharmacol* 62: 913–922.
- Olianas MC, Dedoni S, Onali P (2007). Proteinase-activated receptors 1 and 2 in rat olfactory system: layer-specific regulation of multiple signaling pathways in the main olfactory bulb and induction of neurite retraction in olfactory sensory neurons. *Neuroscience* 146: 1289–1301.
- Patel CB, Noor N, Rockman HA (2010). Functional selectivity in adrenergic and angiotensin signalling systems. *Mol Pharmacol* 78: 983–992.
- Paxinos G, Watson C (1998). *The Rat Brain in Stereotaxic Coordinates*, 4th edn. Academic Press: New York.
- Raffa RB, Connelly CD, Chambers JR, Stone DJ (1996). α -subunit G-protein antisense oligodeoxynucleotide effects on supraspinal (i.c.v.) α 2-adrenoceptor antinociception in mice. *Life Sci* 58: PL77–PL80.
- Remaury A, Larrouy D, Daviaud D, Rouot B, Paris H (1993). Coupling of the alpha 2-adrenergic receptor to the inhibitory G-protein Gi and adenylate cyclase in HT29 cells. *Biochem J* 292: 283–288.
- Rossi GC, Standifer KM, Pasternak GW (1995). Differential blockade of morphine and morphine-6 beta-glucuronide analgesia by antisense oligodeoxynucleotides directed against MOR-1 and G-protein alpha subunits in rats. *Neurosci Lett* 198: 99–102.
- Ruffolo RR Jr, Nichols AJ, Stadel JM, Hieble JP (1991). Structure and function of alpha-adrenoceptors. *Pharmacol Rev* 43: 475–505.
- Scheinin M, Lomasney JW, Hayden-Hixson DM, Schambra UB, Caron MG, Lefkowitz RJ *et al.* (1994). Distribution of alpha 2-adrenergic receptor subtype gene expression in rat brain. *Brain Res Mol Brain Res* 21: 133–149.
- Standifer KM, Rossi GC, Pasternak GW (1996). Differential blockade of opioid analgesia by antisense oligodeoxynucleotides directed against various G protein alpha subunits. *Mol Pharmacol* 50: 293–298.
- Tavares A, Handy DE, Bogdanova NN, Rosene DL, Gavras H (1996). Localization of alpha 2A- and alpha 2B-adrenergic receptor subtypes in brain. *Hypertension* 27: 449–455.
- Wade SM, Lim WK, Lan KL, Chung DA, Namamori M, Neubig RR (1999). G(i) activator region of alpha(2A)-adrenergic receptors: distinct basic residues mediate G(i) versus G(s) activation. *Mol Pharmacol* 56: 1005–1013.
- Wainford RD, Kapusta DR (2009). Chronic high-NaCl intake prolongs the cardiorenal responses to central N/OFQ and produces regional changes in the endogenous brain NOP receptor system. *Am J Physiol* 296: R280–R288.
- Wainford RD, Kapusta DR (2010). Hypothalamic paraventricular nucleus G alpha q subunit protein pathways mediate vasopressin dysregulation and fluid retention in salt-sensitive rats. *Endocrinology* 151: 5403–5414.
- Wainford RD, Kurtz K, Kapusta DR (2008). Central G-alpha subunit protein-mediated control of cardiovascular function, urine output, and vasopressin secretion in conscious Sprague-Dawley rats. *Am J Physiol* 295: R535–R542.
- Watts VJ, Neve KA (2005). Sensitization of adenylate cyclase by Galpha i/o-coupled receptors. *Pharmacol Ther* 106: 405–421.