Angiotensin II Type 2 Receptor Stimulation Initiated After Stroke Causes Neuroprotection in Conscious Rats

Claudia A. McCarthy, Antony Vinh, Brad R.S. Broughton, Christopher G. Sobey, Jennifer K. Callaway, Robert E. Widdop

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Abstract—We have demonstrated previously that pretreatment with an angiotensin II type 2 receptor (AT\(_2\)R) agonist is neuroprotective against a subsequent stroke independent of any changes in blood pressure. Therefore, in the current study, we have examined the potential neuroprotective effect of AT\(_2\)R stimulation initiated after stroke induction to mimic the clinical setting. Intracerebroventricular administration of the AT\(_2\)R agonist CGP42112 was commenced 6 hours after an ischemic stroke had been induced in conscious spontaneously hypertensive rats. CGP42112 given over 4 doses in the same rats (3 \(\mu\)g/kg per dose centrally) at 6, 24, 48, and 72 hours after stroke induction reduced total infarct volume (32±13 mm\(^3\) versus vehicle, 170±49 mm\(^3\); \(P<0.05\)) and improved motor function. Furthermore, we have demonstrated that AT\(_2\)R stimulation after stroke increased neuronal survival, decreased apoptosis, and caused an increase in the number of activated microglia in the core region of damage. The effects of CGP42112 were partially reversed with the coadministration of an AT\(_2\)R antagonist, PD123319. Thus, the current study has shown for the first time that delayed central AT\(_2\)R stimulation after a cerebral incident is neuroprotective in a conscious rat model of stroke. (Hypertension. 2012;60:1531-1537.)

Key Words: angiotensin II type 2 receptors ■ angiotensin ■ stroke ■ hypertension

Angiotensin II (Ang II) can bind to several receptor subtypes, thus, influencing an array of cardiovascular functions in the body. However, the 2 major receptor subtypes are the angiotensin II type 1 receptor (AT\(_1\)R), which is thought to be responsible for most of the biological and pathological actions of Ang II, and the angiotensin II type 2 receptor (AT\(_2\)R), which mainly opposes the actions of the AT\(_1\)R.\(^1\) Several large-scale clinical trials\(^2,3\) have indicated that the use of AT\(_1\)R antagonists as a method of blood pressure control in high-risk patients improves cardiac and vascular events, which results in a reduced risk of secondary events such as stroke. This is consistent with experimental studies reporting that central or peripheral pretreatment with an AT\(_1\)R antagonist reduces postischemic inflammation and the size of the cerebral infarction together with improved behavioral symptoms.\(^4,5\) Importantly, neuroprotection has been demonstrated to occur even with nonhypotensive doses of AT\(_1\)R blockers.\(^6\) The postulated nonvascular mechanisms of neuroprotection include inhibition of neuronal apoptosis\(^7\) and reduced neuronal loss associated with inhibition of superoxide production and inflammation,\(^10\) although there is likely to be a contribution from the AT\(_1\)R during AT\(_1\)R blockade.\(^6,7\) Indeed, several groups have reversed neuroprotection induced during AT\(_1\)R blockade with the coadministration of an AT\(_2\)R antagonist, suggesting that the AT\(_2\)R is a pivotal component in the therapeutic action of AT\(_1\)R antagonists.\(^6,7\) In addition, mice lacking the AT\(_2\)R show poorer outcome after stroke and are less receptive to the protective influence of AT\(_1\)R antagonists.\(^11,12\) Thus, there is accumulating indirect evidence that suggests that the AT\(_2\)R may have a cerebroprotective role during stroke, although little is known about direct AT\(_2\)R stimulation in the brain.

In this context, we have recently examined the neuroprotective effect of direct AT\(_2\)R stimulation after intracerebroventricular (ICV) administration of the AT\(_2\)R agonist CGP42112 in a conscious rat model of stroke. Our results indicate that, even in the absence of AT\(_1\)R blockade, direct stimulation of the central AT\(_2\)R for 5 days before and for 3 days after stroke dramatically reduces subsequent damage, both histologically and behaviorally, such that at 72 hours after stroke injury is minimal.\(^13\) Given that patients normally present for treatment after a cerebrovascular event, a clinically relevant treatment needs to be effective when administered after a stroke has occurred. To this end, the current proof-of-principle study investigated the potential neuroprotective role of...
the AT(R) receptor after central administration of CGP42112, initiated after a stroke has been induced. We hypothesized that CGP42112 would protect neuronal integrity, in part, by decreasing the apoptosis normally associated with stroke.

**Methods**

**Surgical Procedures**

**Cannula Procedures**

Male spontaneously hypertensive rats (SHRs) were anesthetized with a ketamine (75 mg/kg; Sigma)/xylazine (10 mg/kg; Troy; IP), with additional doses administered as required. A 23-gauge stainless-steel guide cannula was stereotaxically implanted into the piriform cortex 3 mm dorsal to the right middle cerebral artery according to a procedure described previously by Sharkey et al.14 The stereotaxic coordinates were modified for this strain of rat (0.2 mm anterior, −4.7 mm lateral, and −7.0 mm ventral relative to Bregma).13 A small amount of ink was injected previously by this intracerebral route to verify correct coordinate placement. In the current investigation, prespecified behavioral indicators were used as an indirect measure of correct cannula placement13 and verified postmortem by assessing the location of the region of damage within the brain. An additional 23-gauge ICV cannula was also stereotaxically implanted into the left lateral ventricle (−0.8 mm anterior, +1.5 mm lateral, and −3.2 mm ventral relative to Bregma). The guide cannulae were secured to the skull with dental acrylic cement and the incision closed with sutures leaving both cannulae exposed. The animals were housed individually and were allowed a 5-day recovery period before the induction of stroke.

**Stroke Induction**

Stroke was induced in conscious animals by titrating the administration of the vasoconstrictor, endothelin-1 (ET-1; Auspep), to cause temporary occlusion of the right middle cerebral artery.13–15 ET-1 was infused via the previously implanted ICV cannula over a period of 3 minutes using a 30-gauge injector protruding 3 mm beyond the end of the guide cannula. The stereotaxic coordinates used for this strain of rat (0.2 mm anterior, −4.7 mm lateral, and −7.0 mm ventral relative to Bregma)13 were used as an indirect measure of correct cannula placement13 and verified postmortem by assessing the location of the region of damage within the brain. An additional 23-gauge ICV cannula was also stereotaxically implanted into the left lateral ventricle (−0.8 mm anterior, +1.5 mm lateral, and −3.2 mm ventral relative to Bregma). The guide cannulae were secured to the skull with dental acrylic cement and the incision closed with sutures leaving both cannulae exposed. The animals were housed individually and were allowed a 5-day recovery period before the induction of stroke.

**Infarct Volume**

Administration of CGP42112 at 6, 24, 48, and 72 hours poststroke significantly reduced both cortical and striatal infarct volume (16.9±10.3 mm³ and 14.6±4.3 mm³, respectively) when compared with vehicle-treated animals (cortical: 133.2±44.2 mm³; striatal: 37.2±6.8 mm³; P<0.05). Thus, total infarct volume was significantly reduced in CGP42112-treated animals (32±13 mm³) compared with vehicle-treated animals (170±49 mm³). Furthermore, this protection was fully reversed with the coadministration of AT(R) antagonist PD123319, such that the combination group was not significantly different from vehicle (Figure 1). PD123319 had no effect when administered alone.

**Motor Deficit**

There was a dramatic increase in percentage error in the ledged beam test in the vehicle-treated SHRs at both 1 and 3 days after stroke (Figure 2). We have reported previously that when given before stroke, CGP42112 significantly reduced the degree of stroke-related deficit at 1 and 3 days after the stroke.13 Similarly, we now demonstrate that CGP42112 reduced the degree of motor deficit at both 1 and 3 days after stroke in the treated animals (11.0±4.4% and 5.2±2.7% error, respectively) when compared with vehicle-treated animals (day 1: 61.0±14.4% and day 3: 58.3±15.8% error; Figure 2). This effect was partially reversed with the coadministration of AT(R) antagonist PD123319, although PD123319 itself reduced motor deficit after stroke (21.7±15.0% error at 3 days; Figure 2).

**Neuronal Survival**

Stroke resulted in a significant reduction in NeuN immunopositive cells in the infarcted region of the ipsilateral hemisphere in vehicle-treated SHRs (10.6±2.9 NeuN-positive cells per millimeter squared), when compared with the noninfarcted region of the same hemisphere (144.4±29.5 NeuN-positive cells per millimeter squared; Figure 3). Administration of CGP42112 after stroke reduced the loss of neuronal cells in the tissue directly affected by stroke (147.5±18.5 NeuN-positive cells per millimeter squared; P<0.001 versus corresponding region in vehicle-treated control). This neuroprotective effect of CGP42112 was fully reversed when PD123319 was coadministered, with both the combination group and PD123319 alone being similar to the vehicle group, exhibiting neuronal loss. No changes were observed in the contralateral hemisphere.
Apoptosis
CGP42112, after stroke, significantly reduced the number of apoptotic cells in the peri-infarct and infarcted regions (peri, 12±3; infarct, 29±5 cleaved caspase-3 positive cells per field of view) in the ipsilateral hemisphere when compared with vehicle treatment (peri, 66±5; infarct, 94±8 cleaved caspase-3 positive cells per field of view; *P<0.01; Figure 4). This anti-apoptotic effect was partially ameliorated when CGP42112 was coadministered with PD123319, whereas PD123319 alone significantly reduced the number of apoptotic cells in both the infarct and peri-infarct regions.

Microglial Activation
As depicted by OX42 immunopositive staining, the number of activated microglial cells in the ipsilateral hemisphere was higher in both core and surrounding regions of the infarct than the noninfarcted tissue (Figure 5A through 5C). There was very little microglial cell activation in the contralateral hemisphere at 3 days after stroke (data not shown). CGP42112, after stroke, significantly increased the number of activated microglia, particularly in the core of the infarct when compared with the corresponding region in vehicle-treated SHRs (44±11 and 14±6 OX42 positive cells per 0.5 mm², respectively; *P<0.05; Figures 5D through 5F and 6), which was fully reversed back to levels in vehicle group when PD123319 was coadministered (Figures 5H through 5J and 6). Interestingly, there was a clear difference in morphological appearance, with an intermediate phenotype showing some processes in the peri-infarct region (Figure 5G) and an amoeboid structure in the core of the infarct (Figure 5K) regardless of the treatment.

Discussion
The key finding of the present study was that direct stimulation of the AT₂R using CGP42112 provided neuroanatomical and behavioral protection when this agent was administered over a 6- to 72-hour period after stroke, independent of any changes in blood pressure, which is consistent with our previous report using a pretreatment protocol.13

In the current study, CGP42112, given after stroke, reduced infarct volume measured at 3 days after stroke and improved motor function at 1 and 3 days poststroke. Indeed, the protective effect of AT₂R stimulation was remarkable,
in that functional motor improvement was observed when initially tested 1 day after stroke, from a single ICV injection of CGP42112 (3 µg/kg per dose) administered 6 hours after ischemia (ie, before the second CGP42112 injection). Furthermore, the histological improvement provided by CGP42112 at 3 days poststroke was reversed with the coadministration of AT₂R antagonist PD123319, indicating that CGP42112 was acting via the AT₂R.

This cerebral ischemic model of stroke is particularly useful because rats were conscious and hypertensive, which mimics the clinical setting while avoiding the confounding neuroprotective effects of anesthesia. The hemodynamic changes induced with the middle cerebral artery application of ET-1 are representative of human stroke, with blood flow reducing at the onset of ET-1, resulting in a complete occlusion of the vessel which resolves over a period of 30 to 40 minutes.

AT₂R stimulation is known to cause systemic and cerebral vasodilatation and has anti-inflammatory effects that may contribute to cerebroprotection, although these mechanisms were not directly examined in the current study. Using the same model, Mecca et al. recently visualized the middle cerebral artery during ET-1 infusion in anesthetized rats and found that acute ET-1-mediated cerebral vasconstriction was unaltered by systemic pretreatment with an AT₂R antagonist, despite the latter causing neuroprotection, although this does not preclude vasodilator effects mediated locally by AT₂R in cerebral microvessels. In the current study, there was no evidence of systemic vasodilatation because blood pressure, measured noninvasively, did not change with drug treatments. Moreover, in the same model, blood pressure measured by radiotelemetry during and 8 hours after stroke did not detect stroke-induced changes in blood pressure between candesartan- and vehicle-treated groups.

Using a neuronal marker (NeuN), we found previously that stroke caused an almost complete loss of neurons in the infarcted tissue and was associated with loss of AT₂R. However, neuronal integrity and AT₂R expression were preserved in CGP42112-treated animals. Similarly, in the current study, when CGP42112 was administered after stroke, the neuronal integrity maintained by CGP42112 was lost with the coadministration of PD123319. Ang II was found previously to increase neuronal viability in primary cortical neurons in a PD123319-reversible manner, which indicates that the AT₂R-mediated neuroprotection can occur independently of cerebral blood flow changes. Moreover, this AT₂R-mediated neuronal preservation is consistent with reduced apoptotic cell death, as evidenced by a marked reduction in cleaved caspase-3 immunopositive cells in CGP42112-treated rats. Thus, in the present study, CGP42112 reduced apoptosis in stroke animals, which reflects greater neuronal preservation with this treatment. Consistent with the current findings, the AT₂R has been associated with increased expression of methyl-methanesulfonate, which plays an important role in DNA repair, protecting against neuronal damage.

A neurotrophic function of the AT₂R is likely to play a major role in the benefit provided by CGP42112, a component of which may involve modulation of microglial activity. Microglia are commonly associated with inflammation after ischemia and were thought to be neurotoxic, although recent evidence supports a neuroprotective role of microglia, by removing cellular debris and infiltrating neutrophils, both of which are a potential source of oxidative damage. As identified by OX42 immunoreactivity
in the current study, there was an increase in the number of activated microglia after stroke, as previously reported using this same stroke model. Additionally, there was also an obvious change in microglia appearance from an amoeboid shape to a phenotype exhibiting processes, when moving from the infarcted core to peri-infarct region (see Figure 5); a finding that has also been demonstrated in different models of stroke in rats and is indicative of phagocytosis of cellular debris. Strikingly, AT2R stimulation after stroke was associated with a significant elevation in the number of activated microglia in the core region of the infarct at 3 days after stroke. CGP42112 is reported to also bind to a non-AT2R site on inflammatory cells, including microglia. However, in the present study, PD123319 fully reversed the CGP42112-induced increased microglia activation, which confirmed that this increase in microglia activity was indeed AT2R mediated. Furthermore, AT2R was upregulated in amoeboid microglial cells after hypoxia. Additionally, it has been reported that exogenously administered microglia migrate to areas of damage to increase neuronal survival. In accordance with the current study, a marked increase in microglia proliferation was seen in mice with small lesion areas after middle cerebral artery occlusion, and this effect was less obvious after a more severe injury, analogous to the (stroked) vehicle-treated animals in the current study. Collectively, our data suggest that microglia can exert a beneficial action on injured neuronal tissue, which is possibly enhanced by AT2R stimulation.

Thus, consistent with our previous pretreatment regime, the current study is the first to demonstrate that, when CGP42112 was commenced 6 hours after an ischemic event, AT2R-mediated neuroprotection was still evident. Whereas the beneficial effects of CGP42112 on infarct size, neuronal integrity, and microglia activation were reversed by PD123319, the AT2R antagonist caused only partial reversal of the effects of CGP42112 on motor function and apoptosis. In this context, Matavelli et al found that PD123319 did not fully reverse the anti-inflammatory effects of compound 21, another AT2R agonist, in the kidneys, which may reflect that current experimental tools are inadequate in some in vivo conditions. Alternatively, it is possible that there was incomplete AT2R blockade using PD123319 in the current dose regime. This differential effect of PD123319 itself seemed to improve motor function and to reduce apoptosis in the current study. Nevertheless, partial agonist activity of PD123319 has not been reported, so the reason for this effect is not entirely obvious, particularly because PD123319 reversed the improvement in motor function caused by CGP42112 when given in a pretreatment regime. Moreover, in our previous study, this AT2R antagonist did not alter motor function when given alone.

Although beyond the scope of the current study, it will be of interest to determine whether direct AT2R stimulation given systemically after stroke also affords neuroprotection. Interestingly, oral administration of the AT2R agonist, compound 21, delayed spontaneous brain abnormalities and extended life expectancy in stroke-prone rats fed a high-salt diet. Although the latter study was not directly examining stroke mechanisms, it demonstrated that chronic pretreatment with compound 21 afforded both renal and neuronal protection that was independent of any changes in blood pressure. Such data are entirely consistent with the current study, whereby stimulation of AT2R 6 to 72 hours after stroke can reduce subsequent damage, thus identifying the AT2R as a potential novel therapeutic target in stroke.

Perspectives

There is an urgent need to develop new therapies that have a larger therapeutic window than tissue plasminogen activator. We have directly stimulated central AT2R after stroke resulting in a dramatic reduction in both cortical and striatal damage. Furthermore, this neuroprotection extended to an improvement in behavioral outcome at both 1 and 3
days poststroke. The neuroprotective mechanisms are likely to be multifaceted and involve increased neuronal survival, reduced apoptotic cell death, AT2R-mediated modulation of the local microglial response, as well as local AT2R-mediated cerebral vasodilatation. Thus, these results provide proof-of-principle for the AT2R as a therapeutic target in the critical window after stroke and therefore should be the focus of future research examining time courses of neuroprotection after systemic administration of AT2R agonists.

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Disclosures

None.

References


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ANGIOTENSIN AT₂ RECEPTOR STIMULATION INITIATED AFTER STROKE CAUSES NEUROPROTECTION IN CONSCIOUS RATS

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Detailed Methods

Animals
Male spontaneously hypertensive rats (SHR), weighing between 250-350g (Animal Resources Centre ARC; Perth) were housed in standard rat cages with a 12-hour light/dark cycle. Food (standard rat chow) and water were provided ad libitum. Experiments were approved by the Monash University, School of Biomedical Sciences Animal Ethics Committee, and were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Systolic blood pressure measurement
Systolic blood pressure (SBP) was measured using a non-invasive tail-cuff apparatus (ADInstruments). An inflatable cuff and pressure transducer were placed around the proximal end of the tail which was then connected to a Mac lab bridge amplifier (ADInstruments, Sydney). A pulsatile signal from the caudal artery was recorded by a Maclab-8 system\(^1\). Animals were placed in clear plastic restrainers and the average of at least 4-5 determinants were calculated on the day prior to commencement of treatment and also 72 hours post-stroke.

Assessment of motor function
Motor coordination and limb function were assessed using the ledged beam as previously described\(^2\). The beam was constructed from wood and consisted of a 1.5m long central section, which tapered from 9cm to 1.5cm from one end to the other. Under-hanging ledges 1.5 cm in width, were positioned 1 cm below the upper surface of the beam. The beam was placed at a 30° angle of incline with the highest point being the narrowest end, which led directly into the animal's home cage. Rats were required to traverse the beam from the widest end to the narrowest, most difficult end. Animals were trained to traverse the beam on the day prior to the pre-treatment assessment. The ledged beam test was conducted immediately before stroke induction, 24 hours post-stroke induction (day 1) and ~78 hours-post stroke induction (day 3). All values were compared to pre-surgery, therefore each rat acted as its own control. The total number of foot steps taken to run the length of the beam was recorded, as well as the number of steps taken on the ledge (errors) by each foot. The latter number was recorded as a percentage of the total number of footsteps taken and recorded as percentage error. It should be noted that the assessment of motor function at 24 hours post-stroke occurred prior to the second injection of the treatment protocol.

Quantification of Ischemic damage
At ~78 hours after stroke, rats were re-anaesthetised and perfused (25 ml/min) with physiologically buffered saline (0.1M PBS; pH 7.4) through the apex of the heart. Brains were removed, frozen over liquid nitrogen, and stored at ~80°C. Coronal cryostat sections (16µm) were cut at 8 pre-determined levels of the forebrain (~3.20mm to 6.8 mm relative to Bregma), slide-mounted and stored at ~80°C. The infarct area and volume were calculated using the ballistic light method developed by Callaway and colleagues that correlates well with thionin staining and light microscopy \(^3\). The area of damage was measured by tracing around the area of infarct in each section using a computerised image analysis system (MCID M4 image analyzer, Imaging Research Inc). The infarct volume was quantified by integrating the
area of damage with the distance between each level \(^4\), providing an estimate of brain injury as reported previously \(^5-7\).

**Immunohistochemistry**

*Neuronal survival*

Neuron integrity was assessed using the neuronal marker NeuN which has been previously used to co-localize the AT\(_2\)R with neuronal cells \(^8\) and to assess neuronal survival after stroke \(^5\). Frozen coronal cryostat sections (16\(\mu\)m) were post-fixed in paraformaldehyde (4\%). Slides were washed in Tris-buffer (Sigma, pH 7.4) for 30 minutes and then incubated in a humidified chamber in 10\% goat serum, 0.5\% BSA and 0.3\% Triton X-100 in Tris-buffer for 1 hour. Sections were then washed in Tris-buffer and incubated overnight in a 1/500 dilution of mouse monoclonal anti-NeuN IgG antibody (Chemicon). Adjacent sections were incubated with the antibody diluting solution, as a negative control. The following day sections were washed in Tris-buffer and incubated for 2.5 hours with the fluorescence labelled secondary antibody, Alexa 488 (1/500 dilution; Invitrogen). Following the incubation period sections were washed once again in Tris-buffer, dried and cover-slipped, using anti-fade mounting reagent (Vector shield) to prevent signal deterioration. The sections were then imaged using a fluorescence imaging microscope. The exact locations of infarcts were determined in each tissue section and these were matched with immunohistochemistry in corresponding tissue sections to ensure that the images were taken from the appropriate infarcted region. The number of NeuN-positive cells was counted by a blinded observer within 1mm\(^2\) sites in the infarcted and non-infarcted regions of the ipsilateral hemisphere and two matched regions of the contralateral hemisphere using analySIS software (Soft Imaging Systems). Two immunopositive sections were analysed per animal.

*Apoptosis*

The number of apoptotic cells was evaluated using an antibody against a common mediator in the apoptotic pathway, cleaved caspase-3. The same procedure was followed which was used to assess neuronal survival, with a few minor changes: tissues were fixed using three 2 min ice cold acetone washes; sections were incubated overnight in a 1/200 dilution of cleaved caspase-3 rabbit polyclonal antibody (AbCAM). For analysis, the number of cleaved caspase-3- immunopositive cells in a 0.5mm\(^2\) area in the peri-infarcted and infarcted regions on the ipsilateral hemisphere was counted by a blinded observer. Two sections were analysed per animal and an average was taken for each animal.

*Microglia activation*

Microglia were detected using an antibody against the CR3 receptor, which has been used by various investigators to visualise microglia in various stages of activation in both lesion and stroke studies \(^9-11\). The same procedure used to detect apoptosis was also used to detect microglia activation, however the primary antibody was mouse monoclonal antibody anti-rat CD11b (1:1000; Serotec), commonly known as OX42. For analysis the number of OX42-immunopositive cells was counted by a blinded observer in a 0.5mm\(^2\) area within the infarcted and non-infarcted region of the ipsilateral hemisphere. Also in the ipsilateral hemisphere, the number of OX42-immunopositive cells in a 0.5mm\(^2\) area was counted in the region bordering the damaged and undamaged tissue or the peri-infarcted region. Ballistic light images from consecutive sections for each individual rat were used to locate the infarct and
non-infarct regions in which cell counts were conducted. As a control measurement, the number of OX42-immunopositive cells in a 0.5mm² area, in matched regions corresponding to the infarct, non-infarct and peri-infarct areas, on the contralateral hemisphere was also calculated.

**Statistical analysis**

Results are presented as mean ± standard error of the mean (SEM). The ledged beam test and systolic blood pressure were analysed using a 2-way ANOVA, with individual differences being detected using a Bonferroni’s post test. Infarct area, neuronal survival and apoptosis were analysed using a 1-way ANOVA, with individual differences being detected by a Bonferroni’s post-hoc test. A value of P<0.05 was considered to be statistically significant; all statistical analyses were performed using GraphPad Prism (GraphPad software).

**References**

Results

Table S1. Effect of Various Treatments on Systolic Blood Pressure (SBP)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pretreatment SBP, mm Hg</th>
<th>72 Hours Post-stroke SBP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (n=5)</td>
<td>164±6</td>
<td>163±5</td>
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<tr>
<td>CGP42112 (3µg/kg/dose; n=10)</td>
<td>164±4</td>
<td>169±4</td>
</tr>
<tr>
<td>CGP42112 (3µg/kg/dose) +</td>
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<td>172±4</td>
</tr>
<tr>
<td>PD123319 (104µg/kg/dose; n=10)</td>
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<tr>
<td>PD123319 (104µg/kg/dose; n=7)</td>
<td>172±5</td>
<td>175±3</td>
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