A Selective Akt Inhibitor Produces Hypotension and Bradycardia in Conscious Rats Due to Inhibition of the Autonomic Nervous System

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Akt is a serine-threonine kinase that is amplified in a variety of human cancers, and as with other anticancer agents, some Akt inhibitors have produced functional cardiovascular effects such as marked hypotension that may limit their clinical benefit. Although identified in preclinical studies, the mechanism(s) responsible for these effects are often not fully characterized; potential targets include Akt signaling disruption in cardiac tissue, vascular smooth muscle, and/or autonomic system signaling. A selective Akt inhibitor was found to produce a rapid and marked hypotension and bradycardia in conscious rats. Isolated right atrial tissue and isolated thoracic aortic rings were used to examine direct effects of Akt inhibition on cardiac and vascular tissues, respectively. In addition, rats surgically prepared with telemetry units for monitoring blood pressure and heart rate were used to investigate potential effects on the autonomic nervous system (ANS). Whereas this Akt inhibitor did not produce any significant effect on atrial tissue, it did cause vasorelaxation of aortic rings. More significantly, in conscious rats, the Akt inhibitor inhibited the neural pressor response to the known nicotinic acetylcholine receptor (nAchR) agonist dimethylphenylpiperazinium (DMPP). In fact, the response observed was comparable to the response observed with the known ganglionic blocker hexamethonium. Thus, the hypotension and bradycardia produced by the Akt inhibitor is primarily due to blockade of nAchRs in autonomic ganglia. This finding highlights the importance of evaluating the ANS for cardiovascular effects associated with new chemical entities as well as suggesting a novel direct effect of an Akt inhibitor on nAchRs.

Key Words: Akt; hypotension; bradycardia; nicotinic acetylcholine receptor; autonomic nervous system; conscious; rat.

Sun et al., 2001), making inhibition of enhanced Akt activity an important therapeutic target for a variety of human cancers (Yap et al., 2008). However, changes in Akt activity could alter functionality in the cardiovascular system due to changes in cardiac contractility, cardiomyocyte size, and L-type Ca2+ current density and Ca2+ flux (Condorelli et al., 2002; Kim et al., 2003).

Changes in cardiovascular function are commonly seen as altered blood pressure regulation (Li, 2007), with profound reductions in arterial pressure reported in a variety of animal models (Zhu et al., 2007). In some cases, the cardiovascular effects observed in animals have also been observed in humans (Harvey and Lonial, 2007; Li, 2007; LoPiccolo et al., 2008; Yap et al., 2008). Other classes of anticancer molecules such as alkylating agents, anthraquinolones, antimicrotubules, and biological agents such as IL-2 and interferon-α2a are also known to produce hypotension and bradycardia in humans (Raschi et al., 2010). Although some cardiovascular effects can be linked to direct effects on the heart or vasculature, through cardiac ion channels or nitric oxide production (Ferro et al., 2004; Kim et al., 2003), not all have been determined. The gold standard preclinical assessment of cardiac safety for a new drug includes evaluation of effects on cardiac structure and function, arrhythmogenic potential, changes in cardiac conduction, and changes in contractility and hemodynamics (Hanton, 2007). In contrast, although crucial to cardiovascular homeostasis, the potential impact of autonomic nervous system (ANS) tone is frequently overlooked as a possible mediator of effects on cardiovascular function (Hanton, 2007).

ANS activation depends on signals from the brain and spinal cord as well as efferent signals from various parts of the body. Transmission of signals in all autonomic ganglia is mediated by nicotinic acetylcholine receptors (nAchRs) comprised of five subunits arranged around a central pore to form a ligand-gated ion channel. nAchRs are triggered by the binding of the locally released neurotransmitter acetylcholine or drug analogs such as nicotine and dimethylphenylpiperazinium (DMPP). The pentameric nAchRs are assembled into various combinations of 12...
distinct subunits (α2–α10 and β2–β4), of which only 6 (α3, α4, α5, α7, β3, and β4) have been shown to exist in autonomic ganglia (Skok, 2002; Wang et al., 2002). The predominant subunit combination found in autonomic ganglia is the α3β4 combination, often referred to as the ganglionic-type nAchR (Skok, 2002). The pharmacologic properties of the different nAchRs is highly dependent on the combination of the α and β subunits (Parker et al., 1998), and therefore, the chemical structure of a drug can allow it to be highly selective for a subtype-specific nAchR.

The studies reported here underscore the importance of the ANS as a controller of cardiovascular homeostasis by demonstrating that a commercially available Akt inhibitor, selective for Akt1 and Akt2, produces hypotension and bradycardia through blockade of nAchRs. That this block is selective for ganglionic nAchRs is seen in its mimicry of hexamethonium, a classic ganglionic blocker, and its ability to completely antagonize the pressor response to the nicotinic agonist DMPP. This novel finding emphasizes the need to consider both direct and indirect mechanisms for assessing cardiovascular liabilities such as hypotension and bradycardia when investigating cardiovascular effects of new chemical entities.

MATERIALS AND METHODS

Reagents and solutions. The Krebs-Henseleit (KH) buffer solution (pH 7.4) used in the isolated rat right atrium and aortic ring assays had the following composition (mM): NaCl 112.0, KCl 4.7, CaCl2 2.5, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25.0, and dextrose 11.0.

The I1 channel blocker (SA nodal inhibitor) 4-ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride (referred to as ZD7288, from Tocris Bioscience, Ellisville, MO) was dissolved in dimethyl sulfoxide (DMSO) and diluted in KH buffer solution to prepare stock concentrations of 0.3, 1, 3, 10, and 30mM. For the isolated rat right atrium assay, 10 μl of each stock concentration was added to the tissue bath chamber to give final concentrations of 0.3, 1, 3, 10, and 30μM. Potassium chloride, carbobal, phenylephrine, and isoproterenol were supplied by Sigma-Aldrich (St Louis, MO) and were dissolved in Millipore water and diluted into KH buffer at final concentrations of 60, 10, 1, and 0.1μM, respectively.

The Akt inhibitor 1,3-dihydro-1-(1-((4-(6-phenyl-1H-imidazo[4,5-g]quinox-7-yl)phenyl)methyl)-4-piperidinyl)-2H-benzimidazol-2-one trifluoroacetate salt hydrate (referred to as Akt-I) was obtained from Sigma-Aldrich. Akt-I has been shown to be a potent and selective allosteric inhibitor of Akt1 and Akt2 with IC50 values of 58 and 210nM, respectively, with no inhibition of Akt1 and Akt2, produces hypotension and bradycardia through blockade of nAchRs. Akt-I has been shown to be a potent and selective allosteric inhibitor of Akt1 and Akt2 with IC50 values of 58 and 210nM, respectively, with no inhibition of other related kinases up to 50μM (Lindsley et al., 2005; Zhao et al., 2005); it produced Akt inhibition in mice at plasma concentrations of 1.5–2.0μM (Lindsley et al., 2005).

For the in vivo experiments, Akt-I solutions were prepared in the hydroxypropyl-β-cyclodextrin Cabivent (ISP Pharmaceuticals, Covington, KY) in sterile water for injection. The Krebs-Henseleit buffer solution (pH approximately 7.4) was used to prepare stock concentrations of 5 and 10 μg/ml. For the isolated rat right atrium and aortic ring assays, Akt-I was dissolved in DMSO and diluted in KH buffer solution to prepare stock concentrations of 10, 30, 100, and 300μM and 1μM. For each of the isolated tissue assays, 10 μl of each stock concentration was added to the tissue bath chamber to give final concentrations of 10, 30, 100, and 300nM and 1μM.

The nAChR selective agonist dimethylphenylpiperazinium (1,1-dimethyl-4-phenylpiperazinium, referred to as DMPP) and the α2-adrenergic agonist phenylephrine hydrochloride (referred to as PE) were supplied by Sigma-Aldrich. DMPP is a ganglionic nAChR agonist that produces an increase in blood pressure and decrease in heart rate; these effects are due to direct stimulation of nAchRs in autonomic ganglia, causing increased sympathetic outflow to blood vessels that raises blood pressure and a decreased parasympathetic outflow to the heart that raises heart rate (Li et al., 2009). Solutions of DMPP and PE were prepared at concentrations of 60 and 10 μg/ml, respectively, in a vehicle composed of 5% dextrose with 0.45% sodium chloride for injection, pH approximately 4.2. The ganglionic blocker hexamethonium bromide (referred to as Hex), which blocks both sympathetic and parasympathetic transmission at preganglionic sites, was supplied by Sigma-Aldrich, and solutions were prepared at a final concentration of 5 mg/ml in the same vehicle. The β1-adrenergic agonist dobutamine hydrochloride (referred to as Dob) was supplied by Hospira, Inc. (Lake Forest, IL) at a stock concentration of 12.5 mg/ml, which was then diluted to a final concentration of 40 μg/ml with sterile saline for injection.

Animals. All animals used in this study were cared for and used in accordance with the requirements outlined by the GlaxoSmithKline Animal Care and Use Committee and in accordance with the Guide for the Care and Use of Laboratory Animals (NRC, 2011). Male Sprague Dawley rats obtained from Charles River Laboratories, Raleigh, NC, were used in this study. The rats were approximately 24–40 weeks of age and weighed between 475 and 600 g during the experiments. Environmental controls were set to maintain temperature within the range 64°F–79°F and relative humidity within the range 30–70%, with an approximate 12-h light and 12-h dark cycle. Rats were offered 23 g of a laboratory diet once daily and except during the periods of restraint, filtered tap water from an automatic watering system was available ad libitum.

Arterial blood pressure and heart rate in conscious rats. Radiotelemetry sensors with a pressure sensitive catheter (Model No. TL1112M-C50-PXT, Data Sciences International [DSI], St Paul, MN) were surgically implanted into rats prior to the start of experiments. For the surgery, animals were anesthetized via inhalation of 3–4% isoflurane in 95% oxygen, a laparotomy was performed and the pressure sensitive catheter was inserted into the abdominal aorta and advanced to a position caudal to the renal bifurcation. The body of the transmitter was secured to the abdominal wall within the peritoneal cavity and the abdomen was closed. Animals were allowed to recover for at least 4 weeks prior to being used for any experiments.

All intravenous infusions were administered via a lateral tail vein catheter that was inserted at least 30 min prior to the collection of baseline measurements. During the periods of time that animals were receiving the intravenous infusions, arterial blood pressure waveforms were recorded in conscious, restrained rats by placing a telemetry receiver (Model RPC-1, DSI) within close proximity of the animal. Following the end of the infusion and restraint period, animals were returned to their home cages to continue to collect arterial pressure and heart rate data. While recording data from the nonrestrained animals, the telemetry receiver was placed within close proximity of the animal’s home cage. The telemetry signals were analyzed using the PO-NE-MAH Physiology P3 software (DSI). Mean arterial, systolic, diastolic and pulse pressures, and heart rate were collected as averages from the nonrestrained animals, the telemetry receiver was placed within close proximity of the animal’s home cage. The telemetry signals were analyzed using the PO-NE-MAH Physiology P3 software (DSI).

Measurement of cardiac response: isolated right atrium. Animals were anesthetized via inhalation of 5% isoflurane in 95% oxygen and euthanized by exsanguination. The chest was opened via a midline incision and the heart was removed and placed in oxygenated (95% O2, 5% CO2) ice-cold KH buffer solution. The right atrium was separated from the ventricles and 5-0 silk suture was tied to opposite ends of the atrium. The tissue was suspended in a 10 ml organ bath containing oxygenated KH buffer solution maintained at 37°C. Each preparation was suspended under a resting tension of 0.5 g and equilibrated for approximately 45 min before the initiation of an experiment. In addition, the bath medium was replaced with fresh prewarmed KH buffer solution every 10 min during the first 30 min of equilibration to remove residual blood and any endogenous transmitters and proteins. The right atrium was allowed to beat spontaneously and the frequency and force of contraction were measured using a force displacement transducer (BioPac TSD125C) and recorded using BioPac software (DA100C control unit and AcqKnowledge software). Only
preparations that exhibited a stable basal frequency of contraction of approximately 220–250 beats/min were considered acceptable for an experiment. Following the 45-min equilibration period, isoproterenol (0.1µM) was added to the bath solution to increase the frequency of contraction to approximately 350–400 beats/min, which is similar to the resting heart rate of a conscious rat. The tissue was allowed to stabilize for approximately 10 min at which time vehicle, Akt-I, or the positive control agent ZD7288 was added to the bath solution. Atrial contraction frequency and force were measured in 5-min intervals for approximately 60 min and the maximal mean interval change was recorded. Data were collected as individual beats and averaged over 30 s for the 5-min measurement periods.

Measurement of vascular response: isolated thoracic aortic rings. Animals were anesthetized via inhalation of 5% isoflurane in 95% oxygen and euthanized by exsanguination. The chest was opened via a midline incision and the thoracic aorta was removed and placed in ice-cold KH buffer solution. The aorta was carefully cleaned to remove excess fat and connective tissue and sectioned into rings approximately 3–4 mm long. The rings were mounted onto tissue clips and suspended in a 10 ml organ bath containing oxygenated KH buffer solution maintained at 37°C. Each ring was suspended under a resting tension of 1 g and equilibrated for approximately 60 min before the initiation of an experiment. In addition, the bath medium was replaced with fresh prewarmed KH buffer solution every 15 min during the first 30 min of equilibration. Following the equilibration period, the viability of the contractile function of each ring was assessed by administering 60µM KCl followed by 1µM PE. To verify the viability and integrity of the endothelium, 10µM carbachol was administered immediately after the PE to produce vasorelaxation. All rings were found to have intact endotheliums and produced the expected responses prior to the initiation of experiments. Following the assessment of vessel integrity and a 45-min equilibration period, the ability of vehicle or Akt-I to induce vasorelaxation was determined by preconstricting each ring with 265nM PE, a concentration previously determined to produce approximately 80% maximal contraction in our laboratory. Once the tension reached a plateau, vehicle or Akt-I was added to the bath. The effect on vasorelaxation was measured for approximately 5 min and the maximal change was recorded.

Measurement of pharmacologic responses in conscious rats: nAChR, α1-adrenergic, and β1-adrenergic agonists. Experiments were conducted as described previously for the measurement of blood pressure and heart rate in conscious restrained rats. In general, after acclimation and a 45-min baseline collection period, animals were infused via a lateral tail vein with either vehicle, Akt-I (50 mg/kg/h), or Hex (10 mg/kg/h). At approximately 120, 150, and 180 min after the start of each infusion, rats were given sequential bolus intravenous injections of DMPP, PE, and Dob of 30, 5 and 20 µg, respectively. Maximal changes in mean arterial pressure and heart rate were calculated as a change from a stable 5 min recording just prior to the injection of DMPP, PE, or Dob. Injections with PE and Dob were only given once the mean blood pressure and heart rate values returned to baseline values (comparable to 5 min recording just prior to administration of the previous agonist).

Statistical analysis. Statistical analysis of the data was performed using a parametric one-way ANOVA model followed by Dunnett’s multiple comparison test. If the parametric assumptions were rejected by the Shapiro-Wilk Statistic and Levine’s test, the data were ranked and then subjected to one-way ANOVA with Dunnett’s multiple comparison test.

RESULTS

Blood Pressure and Heart Rate in Conscious Rats

Blood pressure and heart rate were recorded in conscious rats (n = 4) prior to dosing, during a 1-h iv infusion, and for approximately 24 h afterward. Following the infusion period, animals were returned to their home cages, with blood pressure and heart rate continuously recorded for an additional 22 h. Typical data obtained for up to 5 h following the end of the infusion with either vehicle or 50 mg/kg Akt-I are shown in Figure 1. Whereas the vehicle had no effect on arterial blood pressures or heart rate, a 1-h iv infusion of 50 mg/kg Akt-I dramatically decreased mean arterial blood pressure, which was accompanied by decreased heart rate. The decrease in mean arterial blood pressure was rapid, occurring within 5–10 min after the start of the infusion, and was followed by a decrease in heart rate. The reduction in mean arterial pressure was due to comparable decreases in both systolic and diastolic blood pressures, so pulse pressure was unaltered (data not shown).

As shown in Figure 2, administration of Akt-I at doses of 25 or 50 mg/kg (n = 5 rats per dose) produced sustained dose-dependent reductions in both mean arterial pressure and heart rate. Maximal decreases in mean arterial pressure occurred approximately 2 h after the start of the infusion and maximal decreases in heart rate occurred at approximately 6 h after the start of the infusion. Following a dose of 25 mg/kg, pressure was reduced by approximately 31 mmHg or 24% compared with baseline recordings and heart rate was reduced by approximately 67 beats/min or 17% compared with baseline recordings. Following a dose of 50 mg/kg, pressure was reduced by approximately 53 mmHg, or 36% compared with baseline recordings, and heart rate was reduced by approximately 75 beats/min or 20% compared

FIG. 1. Akt-I (50 mg/kg) or vehicle was administered as a 1-h intravenous infusion as indicated. Data shown represent a typical time course of changes obtained in conscious restrained telemetered rats.
with baseline recordings. The magnitude of reductions in both mean arterial pressure and heart rate due to Akt-I was sustained for at least 6 h after the end of infusion and was still evident 24 h later when compared with vehicle control.

Since maximal decreases in blood pressure and heart rate occurred between 1 and 2 h after the start of dosing, this time period was selected for subsequent experiments.

Contraction Frequency and Force in the Isolated Right Atrium

That administration of Akt-I to conscious rats produced both decreased heart rate and decreased blood pressure suggested concomitant inhibition of the expected baroreflex-mediated pressor response. To determine whether the decreases in blood pressure and heart rate were due to a direct effect of Akt-I on the heart, experiments were conducted using isolated rat right atrial tissue. The right atrium contains the SA nodal cells responsible for the electrical pacing of the heart, and therefore, a direct effect on these cells will alter heart rate. The positive control agent ZD7288 produces bradycardia by directly inhibiting the If pacemaker current through hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in these cells (BoSmith et al., 1993).

The intrinsic contraction frequency of freshly isolated rat right atrial tissue (approximately 220–250 beats/min) was increased to approximately 350–400 beats/min, similar to the resting heart rate of a conscious rat, by incubating the tissue in 0.1μM isoproterenol. When heart rate stabilized, the tissue was then incubated in vehicle, Akt-I, or the positive control agent ZD7288 for 1 h. Each preparation was only exposed to one concentration of vehicle, Akt-I (10nM–1μM), or ZD7288 (1μM). The concentration range for Akt-I was selected based on Akt1 IC50 values of between 0.6 and 14nM for other Akt inhibitors, which produced in vitro cardiovascular effects such as inhibition of hERG current and effects on Purkinje fiber repolarization (Zhu et al., 2007). In addition, in vivo effects such as acute hypotension occur at plasma AUCs of between 1.7 and 2.3μM·h (Zhu et al., 2007). As shown in Figure 3, Akt-I, at concentrations up to 1μM, did not produce any statistically significant changes in contraction frequency or force as compared with vehicle control. In contrast, the known If channel blocker, ZD7288, produced the expected concentration-dependent reductions in the frequency of contraction and increases in force as compared with vehicle control (Nikmaram et al., 1997). Thus, Akt-I–induced bradycardia cannot be explained by a direct effect on cardiac tissue.

Vasorelaxation in Isolated Thoracic Aortic Rings

To examine the potential contribution of direct arterial vasorelaxation induced by Akt-I, experiments were conducted using isolated rat thoracic aortic rings, in which the integrity and responsiveness of the rings were validated as described in the “Materials and Methods” section. Direct vasodilatory properties of Akt-I were examined in aortic rings preconstricted to ~80% maximal with PE, an α1-adrenergic agonist. As shown in Figure 4, Akt-I produced a statistically significant and concentration-dependent relaxation as compared with vehicle treatment alone. Specifically, concentrations of 0.1, 0.3, and 1μM Akt-I produced relaxations of 34, 40, and 47%, respectively, as compared with treatment with vehicle alone, consistent with a contribution by direct vasodilation to the hypotension induced by Akt-I.

Cardiovascular Responses to Challenge with nAchR, α1-Adrenergic, and β1-Adrenergic Agonists in Conscious Rats

In order to explore the potential role of the ANS in Akt-I–induced hypotension and bradycardia, blood pressure and heart rate in conscious rats were again assessed as described earlier but now in the presence/absence of modulators of ANS activity. In general, following the acclimatization and 45 min of baseline data, animals were infused with vehicle, Akt-I, or Hex, a positive control for ganglionic nAchR blockade. Approximately 120, 150, and 180 min later, rats were sequentially challenged with a nAchR agonist, DMPP (30 μg), an α1-adrenergic agonist, PE (5 μg), and then a β1-adrenergic agonist, Dob (20 μg). Figure 5 illustrates a typical response in mean arterial pressure following treatment with vehicle, Akt-I, or Hex and subsequent sequential
administration of DMPP, PE, and Dob. In vehicle control rats, DMPP produced a rapid (within 10 s) and dramatic increase in blood pressure (upper panel) and a subsequent decrease in heart rate (data not shown). As expected, in Hex-treated animals the pressor response to DMPP challenge was inhibited (lower panel).

Figure 6 summarizes the changes in mean arterial pressure and heart rate seen in vehicle-infused, Akt-I–infused, or Hex-infused animals upon DMPP challenge. In vehicle-treated animals, the maximum mean increase in mean arterial pressure was 47 mmHg and the maximum mean decrease in heart rate was 103 beats/min. In Hex-treated animals, the maximum mean increase in mean arterial pressure was only 10 mmHg and the maximum mean decrease in heart rate was only 29 beats/min. In animals infused with 50 mg/kg Akt-I, the pressor response to DMPP challenge was also inhibited. Animals that were given DMPP after receiving Akt-I had mean increases in mean arterial pressure of only 7 mmHg and mean decreases in heart rate of only 12 beats/min.

At approximately 30 min after bolus DMPP challenge, animals were given a 5-μg bolus iv dose of PE (α1-adrenergic agonist); data are summarized in Figure 7. In vehicle control animals, PE produced a rapid (within 5 s) increase in blood pressure and a baroreflex-mediated decrease in heart rate. The maximum mean increase in mean arterial pressure was 67 mmHg and the maximum mean decrease in heart rate was 198 beats/min. In Hex-treated animals, as expected, the pressor response to PE challenge was unaffected, but the baroreflex-mediated decrease in heart rate was depressed with a mean increase in mean arterial pressure of 65 mmHg, and a mean decrease in heart rate of 62 beats/min. In Akt-I–treated animals, both the pressor response to PE challenge and the change in heart rate were diminished as compared with vehicle control. Akt-I–treated animals challenged with PE had mean increases in mean arterial pressure of 28 mmHg and mean decreases in heart rate of 27 beats/min.

Following another 30 min washout (approximately 180 min after the start of the infusion with vehicle, Akt-I, or Hex), rats were given a 20-μg bolus iv dose of Dob (β1-adrenergic agonist); data are summarized in Figure 8. In vehicle control rats, Dob produced a rapid (within 10 s) and dramatic increase in both blood pressure and heart rate. The maximum mean increases in mean arterial pressure and heart rate were 44 mmHg and 75 beats/min, respectively. As expected, in Hex-treated rats, the chronotropic or inotropic response to Dob challenge was unaffected, with mean increases in mean arterial pressure and heart rate of 41 mmHg and 73 beats/min, respectively. Likewise, in Akt-I–treated rats, neither the chronotropic nor the inotropic response to Dob challenge was altered, with mean increases in mean arterial pressure and heart rate of 36 mmHg and 72 beats/min.

**DISCUSSION**

The current study investigated the mechanisms responsible for the hypotension and bradycardia produced in conscious rats
by a potent and selective Akt inhibitor (Akt-I) using both in vivo and in vitro assays as probes for the nature of the target organ for this cardiovascular effect. Akt-I produced dose-dependent reductions in both blood pressure and heart rate, characterized by a rapid onset and extended duration. That the marked decrease in blood pressure was associated with a concurrent decrease in heart rate indicated a possible inhibition of the baroreflex-mediated increase in heart rate. Consequently, experiments were conducted to investigate potential direct effects of the compound on heart and vascular tissue as well as effects on the autonomic system control.

Isolated right atrial tissue and thoracic aortas obtained from Sprague Dawley rats were used to assess direct effects of the Akt-I on cardiac and vascular tissues, respectively. The absence of Akt-I impact on right atrial rate and force of contraction leads to the conclusion that Akt-I does not directly affect cardiac tissue, either SA nodal function or cardiac myocyte contractile force. Therefore, the bradycardia observed in this study is most likely
a result of an effect not mediated by intrinsic mechanisms within the heart. In isolated aortic rings, Akt-I did produce a modest degree of vasodilation, indicating that the compound can produce some direct vasodilation. However, the absence of a concurrent increase in heart rate in the whole animal implies that vasodilation alone is insufficient to explain the cardiovascular effect. Consequently, an indirect target for Akt-I toxicity was considered.

The ANS and, more specifically, autonomic ganglia were considered possible targets for the Akt-I–induced hypotension and bradycardia, in part because the observed time of onset, magnitude, and duration of changes in blood pressure and heart rate induced by Akt-I were similar to that of Hex, an established and specific ganglionic nAChR blocker (Santajuliana et al., 1996). This supposition was supported by the ability of Akt-I to diminish the pressor and rate response to DMPP challenge, an agonist known to be selective for ganglionic nACHRs, especially the \(\alpha_3\beta_4\) and \(\alpha_7\) subunits (Wang et al., 2002). These ganglionic receptor subtypes are highly concentrated in autonomic ganglia in rats (Skok, 2002). The direct vasodilatory effect of Akt-I in isolated aortic rings, the blunted in vivo pressor response to PE challenge, the magnitude of hypotension in vivo, and the lack of a baroreceptor-mediated increase in heart rate argue against angiototoxicity as a primary mechanism. In summary, the profound hypotension and bradycardia induced by Akt-I is primarily mediated by selective block of nAChRs in autonomic ganglia.

The findings from the current study suggest there is value in considering extrinsic (e.g., ANS) factors as well as intrinsic factors when investigating cardiovascular effects associated with new chemical entities. Past evaluation of the hypotensive effects of other Akt inhibitors using a dog femoral artery relaxation assay found little correlation to results obtained in subsequent in vivo cardiovascular dog studies (Zhu et al., 2007), much like the case here in rats. However, by expanding the scope of study to include possible effects on tissue extrinsic to the cardiovascular system, the current study is able to explain both the magnitude and the timing of hypotension induced by this Akt inhibitor. In addition to consideration of species differences in cardiovascular homeostatic control or distinctions in chemical entity structure, selectivity, or specificity, future investigations into effects on cardiovascular function may benefit from utilizing highly selective subunit-specific nAChR agonists and antagonists or functional receptor screens; these may be a more effective, and perhaps necessary approach in the safety evaluation of future novel inhibitors of Akt, or other intracellular signaling paths, for therapeutic use.

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