Inhibition of Smooth Muscle Myosin as a Novel Therapeutic Target for Hypertension

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Received March 29, 2011; accepted July 21, 2011

ABSTRACT

We examined a novel therapeutic approach for hypertension, a small-molecule direct inhibitor of smooth muscle myosin, CK-2018448 (CK-448), which is an *N,N'*-alkylurea (U.S. Patent Publication 2009–0275537 A1) in conscious dogs with renal hypertension and compared its efficacy with that of a calcium channel blocker, amlodipine. Dogs were instrumented with a miniature left ventricular pressure gauge, an aortic pressure catheter, and ultrasonic flow probes in the ascending aorta and renal and iliac arteries for measurement of cardiac output and regional blood flow. In the hypertensive state, mean arterial pressure increased from 101 ± 3.8 to 142 ± 1.9 mm Hg. At the doses selected, CK-448 and amlodipine increased cardiac output similarly ($30 \pm 11\%$ versus $33 \pm 6.4\%$) and similarly reduced mean arterial pressure ($-22 \pm 3.6\%$ versus $-16 \pm 3.4\%$) and total peripheral resistance ($-36 \pm 5.9\%$ versus

$-37 \pm 5.8\%$). CK-448 had the greatest vasodilator effect in the renal bed, where renal blood flow increased by $46 \pm 9.0\%$, versus $11 \pm 3.4\%$ for amlodipine ($p < 0.01$). CK-448 produced significantly less vasodilation in the limb, where iliac blood flow did not change; in contrast, it rose by $48 \pm 12\%$ with amlodipine ($p < 0.01$). The minimal effects on limb blood flow could limit the development of peripheral edema, an adverse side effect of Ca^{2+} channel blockers. In addition, in a rodent model of hypertension, oral administration of a smooth muscle myosin inhibitor resulted in a sustained antihypertensive effect. Thus, the smooth muscle myosin inhibitor's preferential effect on renal blood flow makes this drug mechanism particularly appealing, because many patients with hypertension have renal insufficiency, and patients with heart failure could benefit from afterload reduction coupled with enhanced renal blood flow.

Introduction

Despite the number of available antihypertensive agents that are often used in combination with lifestyle modification, blood pressure remains uncontrolled in 56% of patients treated for hypertension (Lloyd-Jones et al., 2010). Accordingly, there is still a need for novel approaches to the treatment of hypertension. Recently, drugs have been developed that directly target myosin. A direct activator of cardiac myosin has demonstrated promise for the treatment of heart failure (Shen et al., 2010; Malik et al., 2011) and is currently in clinical trials (Cytokinetics, 2007–2009, Greenberg et al., 2009; Habibzadeh et al., 2010).

This study was funded by the National Institutes of Health National Heart, Lung, and Blood Institute [Grants HL0-33107-27, HL0-69020-10, HL10-1420-01, HL0-93481-02, HL10-2472-01, HL0-69752-07], the National Institutes of Health National Institute on Aging [Grant AG0-27211-04], and Cytokinetics, Inc.

Article, publication date, and citation information can be found at <http://jpet.aspetjournals.org>.
doi:10.1124/jpet.111.182402.

Smooth muscle myosin (SMM) is a mechanochemical enzyme that hydrolyzes ATP to generate mechanical force and is responsible for generating vascular tone (Morano et al., 2000; Babu et al., 2001). Inhibitors of myosin function have been described previously (Watanabe et al., 2010). A direct inhibitor of SMM would be attractive for reducing arterial pressure through peripheral vasodilation. A selective, direct smooth muscle myosin inhibitor capable of blocking ATP hydrolysis that relaxes vascular smooth muscle [CK-2018448 (CK-448)] has been developed (Qian et al., 2009b; Clancy et al., 2010), and its effect on vascular resistance in a conscious model of hypertension is now described for the first time.

The goal of the current investigation was to assess the efficacy of this novel therapeutic approach by first examining its effects in conscious dogs, chronically instrumented for instantaneous and continuous measurement of cardiac output, arterial pressure, and blood flow in the renal and iliac beds in the setting of renal hypertension (Vatner et al., 1985).

ABBREVIATIONS: SMM, smooth muscle myosin; CK-448, CK-2018448; CK-509, CK-2018509; MAP, mean arterial pressure; TPR, total peripheral resistance; RR, renal resistance; IR, iliac resistance; LV, left ventricle; SHR, spontaneously hypertensive rat; All, angiotensin II.

A secondary goal was to compare its action with that of a standard antihypertensive drug and known potent vasodilator, which blocks calcium channels, i.e., amlodipine. We were interested in examining the regional vascular effects: the renal bed, because many patients with hypertension have renal insufficiency, and the iliac bed, because peripheral edema, an undesirable side effect of many drugs, is intensified by limb vasodilation, especially with calcium channel blockers (Gustafsson, 1987; Gustafsson et al., 1989; Pedrinelli et al., 2000; Messerli, 2002). The chronically instrumented canine hypertension model permitted monitoring of direct and continuous measurements of regional blood flows and cardiac dynamics, in the absence of anesthesia, which would affect these measurements. The antihypertensive effects of direct SMM inhibitors were examined further using a hypertensive rat model, i.e., spontaneous hypertensive rats (SHRs). This rodent model was particularly useful for examining the effect of another SMM inhibitor, CK-2018509 (CK-509) (Qian et al., 2009a), when given orally on sustained blood pressure lowering.

Materials and Methods

Animals used in this study were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and New Jersey Medical School Institutional Animal Care and Use Committee. Fifteen female mongrel dogs (15–20 kg) were anesthetized with thiopental (15 mg/kg i.v.) followed by endotracheal intubation and halothane (1.0–1.5 vol%) anesthesia. A laparotomy was performed, the left kidney was exposed, and the left renal artery lumen was reduced to a final arterial diameter of 0.9 mm by constricting against a 20-gauge catheter. The right renal artery and left proximal iliac artery were exposed for placement of a Transonic flow probe (Transonic Systems Inc., Ithaca, NY). Through a left thoracotomy Tygon catheters were placed in the descending aorta and the right atrial appendage to measure their respective pressures. A solid-state miniature pressure transducer (Konigsberg, Pasadena, CA) was implanted in the left ventricle (LV) via the apex for LV pressure measurement. Transonic flow probes were placed around the root of ascending aorta to measure cardiac output. The chest and abdomen were closed, and post-operative analgesics were administered until the animals recovered fully from surgery.

SHRs, approximately 12 weeks old, were purchased from Charles River Breeding Laboratories (Portage, MI). For intravenous dosing experiments, rats were anesthetized with isoflurane and femoral artery and vein were cannulated. Drug was administered as a slow bolus over 2 min via the femoral vein. For oral dosing experiments, SHRs were anesthetized and cannula was implanted in the right jugular vein for drug delivery and the right carotid artery for arterial pressure monitoring. Animals were allowed to recover and underwent restrainer acclimatization before oral dosing and blood pressure measurement. For intravenous dosing, CK-2018509 was formulated as a solution in 50% polyethylene glycol 400 and 50% hydroxypropyl β -cyclodextrin. For oral dosing, CK-2018509 was formulated in 10% dimethylacetamide and 90% polyethylene glycol 400 and given by oral gavage. All procedures and protocols were approved by the Cytokinetics, Inc. Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines for the care and use of laboratory animals.

Hemodynamic Measurements. Systemic hemodynamics in dogs were recorded using a signal amplifier (Triton system 6, model 200; Triton Technology, San Diego, CA) and PowerLab data acquisition system (ADInstruments, Inc., Colorado Springs, CO). Aortic and right atrial pressures were measured using strain gauge manometers that had been calibrated with a mercury manometer con-

nected to the fluid-filled catheters. The solid-state LV pressure gauge was cross-calibrated with aortic pressure measurements.

Cardiac output, renal flow, and iliac flow were measured with a Transonic flowmeter (Transonic Systems Inc.; T206). Total peripheral resistance (TPR), renal resistance (RR), and iliac resistance (IR) were calculated: [mean arterial pressure (MAP) – right atrial pressure]/corresponding blood flow. Some flow probes did not work properly, and accordingly, data could not be collected in some cases for the measurements of cardiac output ($n = 2$), renal flow ($n = 2$), and iliac flow ($n = 2$).

In SHRs, blood pressure during intravenous dosing was measured with a solid-state pressure transducer (SPR-320; Millar Instruments Inc., Houston, TX) advanced retrograde via the femoral artery into the descending aorta using a PowerLab data acquisition system (ADInstruments, Inc.). During oral dosing, rats were placed in restrainers for blood pressure monitoring; blood pressure was measured via the carotid artery cannula with a fluid-filled dome pressure transducer (MLT844; ADInstruments, Inc.) using a PowerLab data acquisition system.

Experimental Protocol for CK-448. Before and during the post-operative recovery period, the dogs were trained to lie quietly in the right lateral position. Hemodynamic parameters and arterial pressures were monitored every other day for 2 weeks until mean arterial pressure was consistently elevated to a level higher than 130 mm Hg for at least 3 days, indicating the development of stable hypertension. In four dogs, the arterial pressure did not begin to increase immediately after surgery, and the baseline data from those four dogs were used for the normotensive baseline. To achieve a significant elevation of arterial pressure over baseline (before hypertension), continuous angiotensin II (AII) infusion at a dose of 10 to 15 ng/kg/min was applied in some of the dogs. Before the initiation of the acute experiment, an intravenous catheter (19 gauge) was implanted in the saphenous vein of the hind leg for drug administration. An optimal dose of CK-448, 4 mg/kg i.v. bolus, was selected based on stable dose effects observed during the preliminary dose response study (Fig. 1). To determine whether autonomic reflexes affected the hemodynamic properties, CK-448 was also administered to five normotensive dogs along with autonomic blockade using hexamethonium (30 mg/kg i.v.), propranolol (1 mg/kg i.v.), and atropine (0.1 mg/kg i.v.) bolus. The effects of CK-448 were also compared with those of a known vasodilator used in hypertension therapy, amlodipine. The dose of amlodipine (Pfizer Inc., New York, NY), 0.2 mg/kg i.v., was selected to match the CK-448 dose. Drug responses for CK-448 and amlodipine were continuously monitored for 30 min. Peak responses were analyzed using the averaged data from the first 8 min after drug administration. Vehicle (saline, pH 7.4) was administered to three hypertensive dogs matching the volume of CK-448 administered. CK-448 was provided by Cytokinetics, Inc.

Experimental Protocol for CK-509. SHRs were administered 10 mg/kg i.v. CK-509, and arterial pressures were monitored continuously for 60 min. For oral administration of CK-509, SHRs received a dose of 200 mg/kg on the first day ($t = 0$) and then 50 mg/kg at 24 and 36 h; arterial pressures were monitored for 48 h at discrete time points. In control groups, vehicle (50% polyethylene glycol 400 and 50% hydroxypropyl β -cyclodextrin) for intravenous dosing and oral (10% dimethylacetamide and 90% polyethylene glycol 400) dosing were administered to SHRs via the same methods and in matching volumes as that of CK-509.

Statistical Analysis. Data are expressed as mean $\pm$ S.E. Statistical significance was determined using a two-sample t test. The Wilcoxon rank sum test was used to determine significance in renal flow caused by big variations in the amlodipine group. In the dog experiments, peak response time was chosen based on continuous monitoring, and, accordingly, data from first 8 min after drug administration were averaged.

Dose Response of CK-448 in Conscious Dogs

Fig. 1. Dose response of CK-448 in conscious normal dogs. Cardiac output, renal flow, and iliac flow were compared with CK-448 (2, 4, and 6 mg/kg i.v. bolus; $n = 5$).

Results

Systemic Hemodynamics in Conscious Dogs before and after Hypertension. In the hypertensive state, MAP increased from 101 ± 3.8 to 142 ± 1.9 mm Hg ($p < 0.01$; data not shown), whereas cardiac output fell by $50 \pm 4.9\%$ and TPR rose by $206 \pm 34\%$. LV systolic pressure increased by $37 \pm 3.2\%$, heart rate did not change significantly (96 ± 6.1 to 106 ± 6.9 beats/min), and mean right atrial pressure remained similar between the two groups (4.7 ± 1.4 versus 3.4 ± 0.7 mm Hg; data not shown). Vascular resistances rose in the renal and iliac beds (data not shown).

Antihypertensive Effect of CK-448 Versus Amlodipine. Doses of CK-448 (4 mg/kg i.v. bolus) and amlodipine (0.2 mg/kg i.v. bolus) were selected to decrease MAP and TPR similarly; at these doses heart rate and cardiac output increased similarly with the two drugs at peak response (Table 1; Fig. 2). Peak responses were determined using the averaged values from the first 8 min after drug administration. Administration of vehicle did not have an effect on TPR (data not shown).

Effects on Regional Beds after CK-448 Compared with Amlodipine. CK-448 increased renal flow ($46 \pm 9.0\%$), but did not increase iliac flow (Table 2; Fig. 2). Amlodipine exerted a markedly different effect on the regional beds, where the greatest increase occurred in iliac blood flow ($48 \pm 12\%$), but renal blood flow did not increase much (Table 2; Fig. 2).

CK-448 decreased renal resistance significantly more than amlodipine ($-46 \pm 4.8\%$ versus $-25 \pm 5.4\%$; $p < 0.05$), whereas amlodipine decreased iliac resistance more profoundly than CK-448 ($-42 \pm 5.5\%$ versus $-23 \pm 4.6\%$; $p < 0.05$) (Table 2). Administration of vehicle did not have an effect on the regional beds (data not shown).

The effects of CK-448 were compared in the hypertensive dogs with and without supplemental AII: CK448 increased renal blood flow similarly with (14 ml/min) or without (16 ml/min) supplemental AII, but did not increase iliac blood flow in either group.

Effects of CK-448 after Autonomic Blockade in Normotensive Dogs. After autonomic blockade, CK-448 no lon-

TABLE 1

Hemodynamic measurements after hypertension and during peak drug response

Percentage change from baseline was comprised of the average of the first 8 min after drug administration. Because baseline data before the two drugs were similar, these data were averaged.

	Hypertension Baseline	% Change from Hypertension Baseline	N
Heart rate, beat/min			
CK 448		$34 \pm 4.8^{**}$	9
Amlodipine	112 ± 6.3	$42 \pm 2.2^{**}$	6
Mean arterial pressure, mm Hg			
CK 448		$-22 \pm 3.6^{**}$	9
Amlodipine	141 ± 2.3	$-16 \pm 3.4^{**}$	6
Arterial systolic pressure, mm Hg			
CK 448		$-20 \pm 4.2^{**}$	9
Amlodipine	166 ± 3.5	$-14 \pm 4.8^{**}$	6
Arterial diastolic pressure, mm Hg			
CK 448		$-22 \pm 4.2^*$	9
Amlodipine	129 ± 3.1	-15 ± 5.1	6
LV pressure, mm Hg			
CK 448		$-19 \pm 4.1^{**}$	9
Amlodipine	166 ± 3.5	$-11 \pm 4.0^{**}$	6
LV dP/dt, mm Hg/s			
CK 448		$14 \pm 4.6^*$	9
Amlodipine	3448 ± 152	1.6 ± 6.0	6
Cardiac output, L/min			
CK 448		$30 \pm 11^*$	7
Amlodipine	1.9 ± 0.2	$33 \pm 6.4^{**}$	5
Total peripheral resistance, mm Hg/l/min			
CK 448		$-36 \pm 5.9^{**}$	7
Amlodipine	78 ± 7.6	$-37 \pm 5.8^{**}$	5

* $P < 0.05$, ** $P < 0.01$ from hypertension baseline.

ger increased heart rate and LV dP/dt but reduced mean arterial pressure to a greater extent ($-33 \pm 1.3\%$ versus $-11 \pm 1.4\%$). Although autonomic blockade did not affect the decrease in renal vascular resistance ($-43 \pm 2.9\%$ versus $-39 \pm 1.8\%$; Fig. 3), it did produce greater vasodilation in the iliac bed ($-30 \pm 4.8\%$) compared with the results in the absence of autonomic blockade ($-12 \pm 1.2\%$; Fig. 3).

Antihypertensive Effects of CK-509 in Hypertensive Rats. Unlike CK-448, the SMM inhibitor, CK-509, has the advantage of oral administration. Using SHR, first we dem-

Blood Flow Effects Comparison of CK-448 and Amlodipine

Fig. 2. Time course comparing the effects of amlodipine and CK-448 on cardiac output and renal and iliac artery flows in conscious hypertensive dogs. Percentage changes from baseline (B) are compared between CK-448 and amlodipine for 30 min ($n = 5$, amlodipine; $n = 7$, CK-448). *, $p < 0.05$; **, $p < 0.01$. CK-448 increased renal blood flow, but not iliac blood flow. In contrast, amlodipine increased iliac flow significantly.

TABLE 2

Blood flow effects after hypertension and during peak drug response

Percentage change from baseline was comprised of the average of the first 8 min after drug administration. Because baseline data before the two drugs were similar, these data were averaged.

	Hypertension Baseline	% Change from Hypertension Baseline	N
Renal blood flow, ml/min			
CK 448	48 ± 5.3	46 ± 9.0**††	5
Amlodipine		11 ± 3.4*	7
Renal resistance, mm Hg/l/min			
CK 448	3.7 ± 0.8	-46 ± 4.8**††	7
Amlodipine		-25 ± 5.4**	5
Iliac blood flow, ml/min			
CK 448	105 ± 9.9	0.6 ± 3.1††	5
Amlodipine		48 ± 12*	7
Iliac resistance, mm Hg/l/min			
CK 448	1.5 ± 0.2	-23 ± 4.6**††	7
Amlodipine		-42 ± 5.5**	5

* $P < 0.05$, ** $P < 0.01$ from hypertension baseline.

†† $P < 0.01$ compared with amlodipine.

onstrated that direct intravenous administration of CK-509 (10 mg/kg i.v. bolus) reduced MAP (Fig. 4A). Repeat oral administration of CK-509 produced a sustained reduction in mean arterial pressure over 2 days (Fig. 4B).

Discussion

Despite lifestyle modification, the large number of available pharmacologic agents, and combination therapies, the blood pressure of many hypertensive patients is not well controlled, suggesting the need for novel therapeutic mechanisms. The current investigation of the selective, direct smooth muscle myosin inhibitor CK-448 was conducted in the chronically instrumented conscious dog model of renal hypertension, with continuous measurements of cardiac output, blood flows, and pressures (Knight et al., 1985; Kirby and Vatner, 1987; Gelpi et al., 1988, 1991). This model gave a comprehensive initial picture of the hemodynamic profile of this novel antihypertensive mechanism.

As expected, the SMM inhibitor reduced arterial pressure in both hypertensive dogs and rats. Using the instrumented

large animal model of renal hypertension, we were able to observe not only the decrease in mean arterial pressure but also to measure a decrease in total peripheral resistance and increase in cardiac output. However, the hemodynamic profile was not as predicted based on prior experience. Most vasodilators either affect all peripheral beds similarly (Levine et al., 1984; De Angelis et al., 2005) or elicit preferential vasodilation in the limbs and skeletal muscle (Gavras and Liang, 1980; Mancina and Ferrari, 1992; Shen and Vatner, 1993). The SMM vasodilator we studied exerted an unusual, but potentially desirable, effect on blood flow distribution and regional vasodilation. In particular, this new drug increased renal blood flow markedly by inducing renal vasodilation. Although some of the dogs required supplemental AII infusion to produce hypertension, subgroup analysis showed similarly improved renal blood flow without significant change in iliac blood flow in renal hypertensive animals with or without AII infusion. Furthermore, the renal vasodilation was not affected by autonomic blockade, indicating that the renal vasodilation was a direct effect on the renal vascular bed and not buffered by autonomic reflexes. In contrast, vasodilation in the limb was greater after autonomic blockade, because of alleviation of the reflex constriction in the limb induced by the fall in arterial pressure and unloading of the arterial baroreflex resulting in increased peripheral α -adrenergic vasoconstriction in the limb. The fact that the renal vasodilation was not enhanced after autonomic blockade could be caused by several factors. First, reflex increases in vasoconstrictor tone are generally thought to be less in the kidney than in the limb. It is also possible that the SMM inhibitor blocked renal autoregulation, a powerful mechanism important for maintaining blood flow to the kidney in response to alterations in arterial perfusion by modulating renal vascular smooth muscle tone. Equally important, there was no significant increase in iliac blood flow supplying muscle, bone, and skin, which is favorable, because this adverse effect could intensify peripheral edema (Salmasi et al., 1991; Messerli, 2002).

The second goal of the current study was to compare the effect of the novel SMM vasodilator with another potent

Effects of Autonomic Blockade on CK-448 Responses

Fig. 3. Percentage changes from baseline at 3 min after CK-448 with and without autonomic blockade in conscious normotensive dogs. Mean arterial pressure (MAP), heart rate (HR), TPR, LV dP/dt, RR, and IR were compared in the presence and absence of autonomic blockade. Autonomic blockade eliminated the reflex increases in heart rate and LV dP/dt and induced a greater fall in arterial pressure, but did not affect the reduction in renal vascular resistance ($n = 4$ with blockade group; $n = 5$ without blockade group). **, $p < 0.01$ with blockade different from without blockade.

I.V. and Oral Effect of CK-509
I.V. administration of CK-509 on SHR

Fig. 4. A, effects of CK-509 (10 mg/kg i.v. bolus) in SHRs, showing reduction in MAP ($n = 5$). *, $p < 0.05$ versus vehicle from 1 min after drug administration through 1 h. B, oral administration of CK-509 (200 mg/kg on day 1 followed by 50 mg/kg twice daily) in SHRs ($n = 4$) also reduced MAP over a 2-day monitoring period. *, $p < 0.05$ versus vehicle ($n = 8$) beginning at 1 h of treatment through 48 h.

vasodilator that is commonly used in the treatment of hypertension, a calcium channel blocker, amlodipine. When the two drugs were compared in the hypertensive dogs at doses that increased cardiac output and reduced arterial pressure and total peripheral resistance equally, there was a marked difference in blood flow distribution and regional vascular resistance. The calcium channel blocker had the opposite effect from the SMM inhibitor. The calcium channel blocker did not increase renal blood flow, but increased iliac blood

flow markedly. As mentioned above, this could have the adverse consequences of intensifying peripheral edema, because it is known that this can occur with an increase in limb blood flow (Rendell et al., 1997; Leonetti et al., 2002; Meserli, 2002) and is frequently observed with chronic administration of calcium channel blockers (Rendell et al., 1997; Leonetti et al., 2002). There were no major differences in systemic effects.

Based on the results of the current study, smooth muscle myosin inhibition could be a promising novel strategy for the treatment of hypertension, because these patients should benefit from an increase in renal blood flow. Furthermore, because it did not increase iliac blood flow, it is likely that peripheral edema, an adverse side effect of calcium channel blockers, would not occur with smooth muscle myosin inhibition.

One limitation of the SMM inhibitor used in the canine study is that it is only currently available for intravenous administration, thereby limiting its applicability to the general hypertensive population. However, even in its current intravenous formulation, this novel agent may be suited for use in hypertensive crises or malignant hypertension, when rapid and controlled lowering of arterial pressure is required.

Oral administration is required for therapy in the majority of hypertension patients. Accordingly, we also examined the effects of a different SMM inhibitor, CK-509, which was suitable for oral administration in animal models. The initial studies in SHRs showed that CK-509 administered orally could produce an antihypertensive effect lasting for an entire 2-day observation period.

In conclusion, the preferential increase in renal blood flow with the SMM inhibitor is particularly appealing for hypertension therapy, because many of the patients have renal insufficiency. Future studies will need to establish the poten-

tial benefit of SMM inhibition on end-organ damage in hypertension. Although we did not directly examine the effects of SMM inhibition on renal function, an increase in renal perfusion should be salutary not only in patients with hypertension, but also, in patients with heart failure, particularly those with cardio-renal syndrome, who could benefit from the combination of afterload reduction and increased renal perfusion.

Authorship Contributions

Participated in research design: Zhao, Morgans, Malik, and Vatner.

Conducted experiments: Zhao, Wang, Jia, Pannirselvam, and Vatner.

Contributed new reagents or analytic tools: Morgans and Malik.

Performed data analysis: Zhao, Ho, Dhar, Wang, Jia, Pannirselvam, and Malik.

Wrote or contributed to the writing of the manuscript: Zhao, Ho, Abarzúa, Malik, and Vatner.

References

- Babu GJ, Loukianov E, Loukianova T, Pyne GJ, Huke S, Osol G, Low RB, Paul RJ, and Periasamy M (2001) Loss of SM-B myosin affects muscle shortening velocity and maximal force development. *Nat Cell Biol* **3**:1025–1029.
- Clancy S, Jia Z, Pannirselvam M, Qian X, Morgan B, Malik F, and Hartman J (2010) The small-molecule smooth muscle myosin inhibitor, CK-2018571, selectively inhibits ATP hydrolysis and relaxes smooth muscle in vitro, at the *Biophysical Society 54th Annual Meeting*; 2010 Feb 20–24; San Francisco, CA. Biophysical Society, Rockville, MD.
- De Angelis K, Gama VM, Farah VA, and Irigoyen MC (2005) Blood flow measurements in rats using four color microspheres during blockade of different vasopressor systems. *Braz J Med Biol Res* **38**:119–125.
- Gavras H and Liang C (1980) Acute renovascular hypertension in conscious dogs. Interaction of the renin-angiotensin system and sympathetic nervous system in systemic hemodynamics and regional blood flow responses. *Circ Res* **47**:356–365.
- Gelpi RJ, Hittinger L, Fujii AM, Crocker VM, Mirsky I, and Vatner SF (1988) Sympathetic augmentation of cardiac function in developing hypertension in conscious dogs. *Am J Physiol Heart Circ Physiol* **255**:H1525–H1534.
- Gelpi RJ, Pasipoularides A, Lader AS, Patrick TA, Chase N, Hittinger L, Shannon RP, Bishop SP, and Vatner SF (1991) Changes in diastolic cardiac function in developing and stable perinephritic hypertension in conscious dogs. *Circ Res* **68**:555–567.
- Greenberg BH, Chou W, Saikali KG, Escandon R, Lee JH, Chen M, Malik F, Wolff AA and Shaburishvili T (2009) Phase II safety study evaluating the novel cardiac myosin activator, CK-1827452, in patients with ischemic cardiomyopathy and angina, at the *Heart Failure Society of America Annual Meeting*; 2009 Sept. 13–16; Boston, MA. Heart Failure Society of America, St. Paul, MN.
- Gustafsson D (1987) Microvascular mechanisms involved in calcium antagonist edema formation. *J Cardiovasc Pharmacol* **10**(Suppl 1):S121–S131.
- Gustafsson D, Lanne T, Bjerkhoel P, Johansson P, and Lundvall J (1989) Microvascular effects and edema formation of felodipine in man. *J Hypertens Suppl* **7**:S161–S167; discussion S168.
- Habibzadeh MR, Schiller NB, Malik FI, Chen MM, Saikali KG, Wolff AA, Goldman JH and Teerlink JR (2010) Effect of the selective cardiac myosin activator, omeamtiv mecarbil, on left atrial performance in healthy men, at the *Heart Failure Society of America Annual Meeting*; 2010 Sept 12–15; San Diego, CA. Heart Failure Society of America, St. Paul, MN.
- Institute of Laboratory Animal Resources (1996) *Guide for the Care and Use of Laboratory Animals* 7th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington DC.
- Kirby DA and Vatner SF (1987) Enhanced responsiveness to carotid baroreceptor unloading in conscious dogs during development of perinephritic hypertension. *Circ Res* **61**:678–686.
- Knight DR, Kirby DA, and Vatner SF (1985) Effects of a calcium channel blocker on cardiac output distribution in conscious hypertensive dogs. *Hypertension* **7**:380–385.
- Leonetti G, Magnani B, Pessina AC, Rappelli A, Trimarco B, Zanchetti A, and COHORT Study Group (2002) Tolerability of long-term treatment with lercanidipine versus amlodipine and lacidipine in elderly hypertensives. *Am J Hypertens* **15**:932–940.
- Levine TB, Olivari MT, and Cohn JN (1984) Hemodynamic and regional blood flow response to captopril in congestive heart failure. *Am J Med* **76**:38–42.
- Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, et al. (2010) Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation* **121**:e46–e215.
- Malik FI, Hartman JJ, Elias KA, Morgan BP, Rodriguez H, Brejc K, Anderson RL, Sueoka SH, Lee KH, Finer JT, et al. (2011) Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. *Science* **331**:1439–1443.
- Mancia G and Ferrari AU (1992) Haemodynamic effects of a new multifactorial antihypertensive drug. *Eur Heart J* **13**(Suppl A):26–29.
- Messerli FH (2002) Vasodilatory edema: a common side effect of antihypertensive therapy. *Curr Cardiol Rep* **4**:479–482.
- Morano I, Chai GX, Baltas LG, Lamounier-Zepter V, Lutsch G, Kott M, Haase H, and Bader M (2000) Smooth-muscle contraction without smooth-muscle myosin. *Nat Cell Biol* **2**:371–375.
- Pedrinelli R, Dell’Omo G, Melillo E, and Mariani M (2000) Amlodipine, enalapril, and dependent leg edema in essential hypertension. *Hypertension* **35**:621–625.
- Qian X, Chuang C, Lu P, Yao B, Lu Q, Jiang H, Wang W, Morgan BP, and Morgans DJ (2009a) inventors; Cytokinetics Inc., assignee. Certain chemical entities, compositions, and methods. U.S. Patent Publication No. US 2009–0275537 A1; 2009 Nov 5.
- Qian X, Wang X, Hartman J, Jia Z, Clancy S, Yao B, Chuang C, Lu P, Pannirselvam M, Morgans D, et al. (2009b) A direct inhibitor of smooth muscle myosin as a novel therapeutic approach for the treatment of systemic hypertension, at the *American Society of Hypertension 24th Annual Scientific Meeting and Exposition*; 2009 May 6–9; San Francisco, CA; American Society of Hypertension, New York.
- Rendell MS, Shehan MA, Kahler K, Bailey KL, and Eckermann AJ (1997) Effect of calcium channel blockade on skin blood flow in diabetic hypertension: a comparison of isradipine and atenolol. *Angiology* **48**:203–213.
- Salmasi AM, Belcaro G, and Nicolaides AN (1991) Impaired venoarteriolar reflex as a possible cause for nifedipine-induced ankle oedema. *Int J Cardiol* **30**:303–307.
- Shen YT, Malik FI, Zhao X, Depre C, Dhar SK, Abarzúa P, Morgans DJ, and Vatner SF (2010) Improvement of cardiac function by a cardiac myosin activator in conscious dogs with systolic heart failure. *Circ Heart Fail* **3**:522–527.
- Shen YT and Vatner SF (1993) Effects of a K⁺ATP channel opener, lemakalim, on systemic, coronary and regional vascular dynamics in conscious dogs: comparison with nifedipine, adenosine, nitroglycerin and acetylcholine. *J Pharmacol Exp Ther* **265**:1026–1037.
- Vatner DE, Kirby DA, Homcy CJ, and Vatner SF (1985) β -Adrenergic and cholinergic receptors in hypertension-induced hypertrophy. *Hypertension* **7**:155–160.
- Watanabe M, Yumoto M, Tanaka H, Wang HH, Katayama T, Yoshiyama S, Black J, Thatcher SE, and Kohama K (2010) Blebbistatin, a myosin II inhibitor, suppresses contraction and disrupts contractile filaments organization of skinned taenia cecum from guinea pig. *Am J Physiol Cell Physiol* **298**:C1118–C1126.

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