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REVIEW

The vasodilatory action of testosterone: a potassium-channel opening or a calcium antagonistic action?

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Abbreviations: 4-AP, 4-aminopyridine; 5β-DHT, 5β-dihydrotestosterone; 5-HT, 5-hydroxytrypamine; AR, androgen receptor; $BaCl_2$, barium chloride; BK_{Ca}, calcium sensitive maxi-potassium channel; BSA, bovine serum albumin; BAY, BAY K8644; $[Ca^{2+}]_i$, intracellular calcium concentration; CAD, coronary artery disease; Caff, caffeine; Carb, carbachol; CBF, coronary blood flow; cGMP, cyclic guanosine monophosphate; ChTX, charybdotoxin; DAG, diacyl glycerol; ER, oestrogen receptor; IBTX, iberiotoxin; IP_3, inositol triphosphate; IP_3R, inositol triphosphate receptor; K_{ATP}, ATP-sensitive potassium channel; K_{Ca}, calcium-sensitive potassium channel; K_V, voltage-sensitive potassium channel; KCl, potassium chloride; Meth, methoxamine; NA, noradrenaline; NO, nitric oxide; PDBu, phorbol dibutyrate; $PDBu^{-Ca}$, phorbol dibutyrate in calcium free saline; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PKC, protein kinase C; PLC, phospholipase C; ROCC, receptor-operated calcium channel; RyrR, ryanadine receptor; SHBG, sex hormone binding globulin; SOCC, store-operated calcium channel; TBA, tetrabutylammonium; TEA, tetraethylammonium; Thap, thapsigargin; VOCC, voltage-operated calcium channel

Testosterone has classically been considered to exert a detrimental influence upon the cardiovascular system due to the high male prevalence of coronary artery disease (CAD), which even after correcting for sex-dependent differences in risk factors, remains double that in women (Wingard *et al*., 1983; Jousilahti *et al*., 1999). However, numerous cross-sectional case control studies report hypotestosteronaemia in patients with heart disease (reviewed in Alexandersen *et al*. (1996)) and a recent study by our group has demonstrated that men with significant CAD have markedly reduced circulating levels of testosterone (English *et al*., 2000c). Similarly, testosterone levels are reported to be inversely proportional to atherosclerotic risk (Khaw & Barrett-Connor, 1991; Duell & Bierman, 1990; Hromadova *et al*., 1991), and other risk factors for CAD including hypertension, hyperinsulinaemia, diabetes, obesity, smoking, age and an adverse thrombotic profile are all associated with hypotestosteronaemia (reviewed in English *et al*., 1997). Such observations contradict this traditional view and suggest that testosterone is beneficial to the cardiovascular system, at least in male individuals, and that it is a relative deficiency in circulating testosterone which is associated with coronary heart disease.

Indeed, a number of studies support this hypothesis. Research dating back to the 1940s has demonstrated a beneficial effect of testosterone therapy in male patients with angina (Hamm, 1942; Walker, 1942; Sigler & Tulgan, 1943; Lesser, 1946), although it was a further 30 years until the first placebo-controlled data was provided: Jaffe (1977) reported an improvement in myocardial ischaemia in men who had received either four or eight, weekly intramuscular testosterone injections. Subsequent studies have demonstrated that chronic administration of high dose oral testosterone (Wu & Weng, 1993), and physiological trans-dermal testosterone (English *et al*., 2000d), also improve symptom scores of angina and reduce objective measures of myocardial ischaemia. Furthermore, acute administration of testosterone also provides rapid improvements in myocardial ischaemia (Webb *et al*., 1999a; Rosano *et al*., 1999), prompting the suggestion that testosterone may beneficially influence coronary vascular tone. Indeed, in subsequent studies Webb *et al*. (1999b) demonstrated that an intra-coronary infusion of physiological concentrations of testosterone increased coronary artery diameter and coronary blood flow in male patients with CAD, consistent with a direct coronary vasodilatory action.

Numerous animal studies have also demonstrated that testosterone acts as a direct coronary vasodilator in a variety of species, including rabbit, dog, pig and rat, both *in vivo* (Chou *et al*., 1996) and *in vitro* (Yue *et al*., 1995; Murphy & Khalil, 1999; Crews & Khalil, 1999a; English *et al*., 2000b; 2002; Deenadayalu *et al*., 2001). The beneficial effects of testosterone upon myocardial ischaemia are attributed to this activity. Testosterone is also reported to exhibit a vasodilatory action in thoracic aortae (Yue *et al*., 1995; Perusquia *et al*., 1996; Honda *et al*., 1999; Ding & Stallone, 2001) and in vessels isolated from the mesenteric (Tep-areenan *et al*., 2002) and pulmonary (English *et al*., 2001; Jones *et al*., 2002a)

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vasculature. Furthermore, recent reports demonstrate that both acute administration of intravenous testosterone (Ong *et al*., 2000) and chronic administration of oral testosterone (Kang *et al*., 2002) enhance brachial artery responsiveness to flow and nitrate-mediated dilatation in men with CAD. Such observations demonstrate that in addition to a direct vasodilatory action, testosterone also exerts a beneficial effect upon the regulation of vascular tone controlled by the release of nitric oxide from the endothelium, and in the clinical setting in which such therapy could be employed – male patients with significant CAD.

Mechanisms of testosterone-induced vasodilatation

Involvement of the androgen receptor

Numerous studies have been conducted to elucidate the dilatory mechanism of action of testosterone. Classically testosterone is recognized to regulate cellular function *via* interaction with the nuclear androgen receptor (AR). Testosterone (together with the other sex hormones) is transported in the bloodstream in conjunction with a binding protein; either sex hormone binding globulin (SHBG) to which it is tightly bound, or albumin, to which testosterone has a much lower affinity. At the target cell testosterone dissociates from the binding protein, undergoes endocytosis, and in most cell types is converted to dihydrotestosterone prior to binding to the nuclear AR. The hormone-receptor complex then directly interacts with the nuclear DNA, initiating protein synthesis. This process takes hours in most instances, although some genomic effects have been identified to occur in 40 min.

The initial focus of studies investigating the vasodilatory mechanism of action of testosterone, was the degree of involvement this classical genomic signalling pathway played in the response (summarized in Table 1). The observation that testosterone-mediated vasodilatation is evident within minutes of application and is maximal by 20 min, is strongly suggestive that activation of protein synthesis is not a pre-requisite for vascular relaxation. However, more substantial evidence is provided by findings that testosterone-induced dilatation is not attenuated either by pre-treatment with the AR blocker flutamide (Yue *et al*., 1995; Tep-areenan *et al*., 2002; Jones *et al*., 2002a), or by the covalent linkage of testosterone analogues to albumin, thus preventing endocytosis into the smooth muscle cell (Ding & Stallone, 2001; English *et al*., 2000a). Furthermore, polar, non-permeable testosterone analogues have been shown to elicit greater vasodilatation than non-polar, permeable analogues (Ding & Stallone, 2001), and non-genomic testosterone analogues have also been shown to elicit greater vasodilatation than genomic-acting analogues (Yue *et al*., 1995; Ding & Stallone, 2001). Research from our laboratory has also demonstrated that testosterone-mediated vasodilatation is maintained in vessels isolated from testicular feminized mice, which lack a functional androgen receptor (Jones *et al*., 2002b).

The observation that testosterone is able to regulate cellular activity apparently independently of the classical signalling pathway is not restricted to the vascular smooth muscle. Rapid actions of testosterone are reported in a variety of cell types (Table 2), and such observations have spurred the suggestion that testosterone may also activate alternative, non-genomic signalling pathways, and that cell surface ARs may also exist. The subject of non-genomic signal transduction is outside the scope of the present article but is extensively covered in the excellent review of Falkenstein *et al*. (2000). However, numerous studies utilizing a variety of diverse cell types demonstrate rapid actions of testosterone upon calcium signalling (Table 2), which clearly has implications for vascular smooth muscle.

Local conversion to 17β oestradiol

17β oestradiol is also recognized to elicit marked vasodilatation in a variety of vascular beds (Chester *et al*., 1995; Browne *et al*., 1999; Salom *et al*., 2001; English *et al*., 2001), and since testosterone is readily converted into 17β oestradiol *via* the enzyme aromatase, this presents another potential mechanism by which testosterone may induce vascular relaxation. However evidence from a number of studies (Table 1) precludes such an action: Vasodilatation to testosterone is not reduced by either aromatase inhibition (Yue *et al*., 1995; Tep-areenan *et al*., 2002) or oestrogen receptor antagonism (Chou *et al*., 1996), and similar vasodilation is triggered by non-aromatizable dihydrotestosterone (Deenadayalu *et al*., 2001). Furthermore, in the pulmonary vasculature, the vasodilatory efficacy of 17β oestradiol is significantly lower than testosterone (English *et al*., 2001) and consequently aromatase-mediated conversion of testosterone into 17β oestradiol is unlikely to be involved in the response in this vascular bed.

Involvement of endogenous vasodilators

The vasodilatory action of testosterone is also reported to be independent of the release of dilator prostaglandins and of endothelial-derived dilatory agents such as endothelial derived relaxing factor (nitric oxide – NO) or endothelial derived hyperpolarising factor (summarized in Table 3).

A number of studies have investigated the involvement of dilator prostanoids in the response to testosterone by treatment with indomethacin, an inhibitor of cyclo-oxygenase, the enzyme responsible for the synthesis of the cyclic endoperoxide prostaglandin precursors from arachindonate. However, all such studies report that indomethacin has no inhibitory effect upon testosterone-mediated relaxation (Yue *et al*., 1995; Chou *et al*., 1996; Honda *et al*., 1999; Jones *et al*., 2002a). Similarly the majority of studies have demonstrated that testosterone-induced dilatation is preserved in endothelial denuded vessels (Yue *et al*., 1995; Honda *et al*., 1999; Deenadayalu *et al*., 2001; Perusquia *et al*., 1996; Perusquía & Villalón, 1999; Crews & Khalil, 1999a, b; Murphy & Khalil, 1999) or in the presence of inhibitors of nitric oxide synthase or guanylate cyclase (Yue *et al*., 1995; Honda *et al*., 1999; Deenadayalu *et al*., 2001; Jones *et al*., 2002a). Even in the few studies that do demonstrate a reduction in the efficacy of testosterone following such interventions (Chou *et al*., 1996; Ding & Stallone, 2001; Tep-areenan *et al*., 2002), the observed attenuation is modest, with a sizeable portion of the vasodilatory response remaining. Consequently additional dilatory mechanisms must also be involved.

Table 1 Studies investigating the influence of the androgen receptor or aromatase-mediated conversion to 17β oestradiol in the vasodilatory mechanism of action of testosterone

Species	*Preparation*	*Contractile agonist*	*Concentration range of testosterone*	*Incubation*	*Effect*	*Conclusion*	*Reference*
Rabbit	Coronary arteries and thoracic aorta 1 g isometric tension	$PGF_{2\alpha}$ (3 μM)	1–10 μM	None Flutamide (10 μM) AmGlut (50 μM)	95% relaxation $\leftrightarrow$ $\leftrightarrow$	Dilatation does not involve aromatase or the AR.	Yue *et al*. (1995)
Dog	Conduit and resistance coronary arteries *in vivo*	None	100 nM–1 μM	None ICI 182780 (10 μM)	86%$\uparrow$ in CBF $\leftrightarrow$	Dilatation does not occur *via* the oestrogen receptor.	Chou *et al*. (1996)
Rat	Thoracic aortae 2.5 g isometric tension	NA (1 μM)	5–300 μM	THS BSA-linked THS	85% relaxation $\uparrow$	Dilatation does not occur *via* the nuclear AR.	Ding & Stallone (2001)
Rat	Mesenteric arterial bed Isobaric tension 15 mmHg	Meth (25 μM) Meth (178 μM)	100 pM–10 μM	None Flutamide (10 μM) AmGlut (50 μM)	68% relaxation $\uparrow$ $\uparrow$	Conversion to 17β oestradiol occurs but does not contribute to dilatory action. The AR may also act to reduce testosterone bio-availability but does not contribute to the dilatory action.	Tep-areenan *et al*. (2002)
Rat	Pulmonary arteries Isometric tension equivalent to 17.5 mmHg	$PGF_{2\alpha}$ (100 μM)	1–1000 μM	None Flutamine (10 μM)	30% relaxation $\uparrow$	The AR amy act to reduce testosterone bio-availability but does not contribute to the dilatory action.	Jones *et al*. (2002)

List of abbreviations: AmGlut = aminoglutethimide; AR = androgen receptor; BSA = bovine serum albumin; CBF = coronary blood flow; Meth = methoxamine; NA = noradrenaline; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; THS = testosterone hemisuccinate; $\leftrightarrow$ = no change in efficacy; $\uparrow$ = small increase in efficacy.

However, evidence does support a modulatory action for testosterone upon endothelial function. Testosterone therapy has been demonstrated to improve flow-mediated brachial artery dilatation which occurs *via* sheer-stress-induced release of NO (Ong *et al*., 2000; Kang *et al*., 2002). Such data indicate that whilst testosterone does not induce vasodilatation solely through direct NO release, it clearly is able to sensitize the smooth muscle to the actions of NO. Indeed, a reduced circulating testosterone profile is associated with a reduced efficacy of acetylcholine in the Tfm mouse (Jones *et al*., 2002b), whilst exogenous testosterone therapy is reported to improve dilatation to nitroglycerin (Kang *et al*., 2002). This underlying action is as yet unknown but may be at the level of the cGMP/protein kinase G axis (Deenadayalu *et al*., 2001).

Whilst the results of these studies demonstrate some interaction of testosterone upon endothelial cell signalling, the majority of the vasodilatory action of testosterone is effected by a direct action on the vascular smooth muscle. At present, experiments aimed at unearthing the mechanism underlying this response support two contradictory hypotheses, (i) potassium channel activation or (ii) calcium channel antagonism.

Testosterone as a potassium channel opening agent

A number of studies have provided evidence of a modulatory role for testosterone upon potassium channel function (Table 4), the first being that of Yue *et al*. (1995), utilizing isolated rabbit coronary arteries and thoracic aortae preconstricted with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). After excluding the involvement of endogenous dilatory pathways Yue *et al*. (1995) demonstrated that testosterone had no inhibitory effect upon the contraction induced by addition of calcium to the organ bath under depolarizing conditions, responses which were sensitive to the voltage-operated calcium channel (VOCC) blocker verapamil. Furthermore, whilst the vasodilatory action of testosterone was unaffected by incubation with the ATP-sensitive potassium channel (K_{ATP}) blocker glibenclamide, testosterone-induced vasodilatation was reduced

Table 2 Studies demonstrating rapid effects of testosterone upon calcium homeostasis in a variety of cell types, indicative of signal transduction mediated *via* non-genomic cell surface receptors

Species	*Cell type*	*Observation*	*Reference*
Rat	Osteoblasts	Testosterone resulted in a rapid increase in the concentration of intracellular Ca^{2+} (5s) and subsequently IP_3 and DAG (10s), in male rat osteoblasts. All responses were insensitive to AR blockade *via* cyproterone acetate and the covalent linkage of testosterone to BSA, but could be abolished by inhibiting PLC.	Lieberherr & Grosse (1994)
Mouse	T lymphocytes	Testosterone induced a rapid (10 s) rise in the intracellular free Ca^{2+} concentration in activated T cells, which was insensitive to AR blockade with cyproterone acetate and the covalent linkage of testosterone to BSA. The response was inhibited by Ni^{2+} and removal of extracellular calcium, indicative of testosterone-mediated activation of non-voltage sensitive calcium channels.	Benten *et al*. (1997)
Mouse	IC-21 Macrophages	Testosterone induced a rapid (10 s) rise in the concentration of intracellular Ca^{2+} in the macrophage cell line IC-21 which lack an intracellular AR. The response was insensitive to the AR blockers flutamide and cyproterone acetate, and the covalent linkage of testosterone to BSA. The response was abolished by the PLC inhibitor U-73122, indicating the prerequisite for production of IP_3 and DAG, and suggesting the source of calcium to be the intracellular stores.	Benten *et al*. (1999)
Rat	Sertoli cells	Testosterone resulted in a rapid (2 min) increase in the intracellular free Ca^{2+} concentration, which was insensitive to the nuclear AR antagonist cyproterone acetate and the covalent linkage of testosterone to BSA. The response was abolished in the absence of extracellular calcium and significantly reduced by the VOCC blocker verapamil.	Lyng *et al*. (2000)
Human	Prostatic tumour cells	Testosterone induced a rapid (2 min) rise in the intracellular free Ca^{2+} concentration in the prostatic tumour cell lines LNCaP (which contain a mutated nuclear AR) and PC3 (which lack a nuclear AR). The elevation in intracellular calcium was similar in response to free testosterone and testosterone bound to BSA, was abolished by the absence of extracellular calcium and significantly reduced by the VOCC blocker verapamil.	Lyng *et al*. (2000)
Rat	Skeletal myocytes	Testosterone triggered a rapid elevation in intracellular Ca^{2+} (10–15 s) and IP_3 (45 s) in embryonic myotubes, which was insensitive to cyproterone acetate but reduced in the absence of extracellular calcium.	Estrada *et al*. (2000)
Mouse	RAW 264.7 macrophages	Testosterone induced a rapid (10 s) rise in the intracellular free Ca^{2+} concentration in RAW 264.7 macrophages which lack an intracellular AR. The response was unaffected by AR and ER blockade, was similar upon exposure to both free and BSA-linked testosterone, and did not result in activation of the ERK1/2, p38 or JNK/SAPK signalling pathways or in the production of NO.	Guo *et al*. (2002)

List of abbreviations: AR = androgen receptor; BSA = bovine serum albumin; DAG = diacyl glycerol; ER = oestrogen receptor; IP_3 = inositol triphosphate; NO = nitric oxide; PLC = phospholipase C; VOCC = voltage operated calcium channel.

by incubation with barium chloride ($BaCl_2$), and in vessels pre-constricted with potassium chloride (KCl). $BaCl_2$ acts as a non-specific inhibitor of potassium channels, and in the presence of high concentrations of extracellular potassium ions (as generated by the addition of millimolar concentrations of KCl), the residual intracellular-extracellular flux of potassium ions which occurs *via* membranous voltage-sensitive potassium channels (K_V), calcium-sensitive potassium channels (K_{Ca}) and K_{ATP} channels is inhibited (Figure 1). Consequently under both these experimental conditions potassium channel function is compromised, in conjunction with the vasodilatory efficacy of testosterone. Yue and colleagues therefore concluded that testosterone-mediated relaxation occurred through potassium channel activation, and since the glibenclamide data precluded involvement of K_{ATP} channels, this was likely to be *via* activation of K_{Ca} and/or K_V channels.

In contrast, activation of K_{ATP} channels is proposed to contribute to testosterone-induced dilatation in two subsequent studies. Chou *et al*. (1996) assessed the vasodilatory action of testosterone *via* the measurement of the *in vivo* changes in coronary blood flow (CBF) by simultaneous intravascular two-dimensional and Doppler ultrasound, and reported that the testosterone-induced increase in CBF was significantly reduced by a preceding infusion of glibenclamide (Chou *et al*., 1996). However the efficacy of glibenclamide was restricted to the coronary resistance vessels, which may help explain the discrepancy with the study of Yue *et al*. (1995) which utilized conduit coronary arteries. Honda *et al*. (1999) studied the vasodilatory mechanism of testosterone in thoracic aortae isolated from normal and spontaneously hypertensive rats. Again in contrast to Yue *et al*. (1995), vasodilatation to testosterone was significantly attenuated in the vessels harvested from normotensive animals by incubation with the K_{ATP} channel blocker glibenclamide. However in the vessels obtained from hypertensive animals, the response to testosterone was again reduced by glibenclamide, but also by the K_{Ca} channel blocker tetraethylammonium (TEA) and the K_V channel blocker 4-aminopyridine (4-AP). The results of this study are interesting since they demonstrate that in the hypertensive setting testosterone influences the function of additional potassium channels,

Table 3 Studies investigating the influence of the endothelium and dilator prostaglandins in the vasodilatory mechanism of action of testosterone

Species	*Preparation*	*Contractile agonist*	*Concentration range of testosterone*	*Incubation*	*Effect*	*Conclusion*	*Reference*
Rabbit	Coronary arteries and thoracic aorta 1 g isometric tension	$PGF_{2\alpha}$ (3 μM)	1–10 μM	None Endothelial denudation Methylene blue (10 μM) L-NAME (100 μM) Indomethacin (10 μM)	95% relaxation ↔ ↔ ↔ ↔	Dilatation is endothelium, cGMP, NO and dilator Prostanoid independent.	Yue *et al.* (1995)
Dog	Conduit and resistance coronary arteries *in vivo*	None	100 nM–1 μM	None L-NAME (100 μM) Indomethacin (5 mg kg^{-1})	86%↑ in CBF ↓ ↔	Dilatation is partially NO-dependent.	Chou *et al.* (1996)
Rat	Thoracic aortic 1 g isometric tension	NA (0.3 μM)	30 μM	None Endothelial denudation	100% relaxation ↔	Dilatation is endothelium independent	Perusquía *et al.* (1996)
Rat	Thoracic aortic 0.7 g isometric tension	NA (300 nM)	9–300 μM	None Endothelial denudation L-NAME (100 μM) Indomethacin (10 μM)	95% relaxation ↔ ↔	Dilatation is endothelium, NO and dilator prostanoid independent.	Honda *et al.* (1999)
Rat	Thoracic aortae 2.5 g isometric tension	NA (1 μM)	5–300 μM	None Endothelial denudation	100% relaxation ↓	Dilatation is partially NO-dependent.	Ding & Stallone (2001)
Pig	Coronary arteries 2.5 g isometric tension	$PGF_{2\alpha}$ (10 μM)	5–75 μM	None Endothial denudation L-NAME (250 μM)	97% relaxation ↔ ↔	Dilatation is endothelium and NO independent.	Deenadayalu *et al.* (2001)
Rat	Mesenteric arterial bed Isobaric tension 15 mmHg	Meth (25 μM) Meth (3 μM)	100 pM–10μM	None Endothelial denudation L-NAME (300 μM)	68% relaxation ↓ ↓	Dilatation is partially NO-dependent.	Tep-areenan *et al.* (2002)
Rat	Pulmonary arteries Isometric tension equivalent to 17.5 mmHg	$PGF_{2\alpha}$ (100 μM)	1–1000 μM	None L-NAME (10 μM) Indomethacin (10 μM)	30% relaxation ↔ ↔	Dilatation is NO and dilator prostanoid independent.	Jones *et al.* (2002)

List of abbreviations: CBF = coronary blood flow; cGMP = cyclic guanosine monophosphate; KCl = potassium chloride; L-NAME = N^{ω}-nitro-L-arginine methyl ester; Meth = methoxamine; NA = noradrenaline; NO = nitric oxide; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; ↔ = no change in efficacy; ↓ = small reduction in efficacy.

which may be a consequence of an alteration in channel expression in the disease state. However, the authors provide little explanation for the discrepancy with the study of Yue *et al.* (1995) in the vasodilatory mechanism of action of testosterone under normotensive conditions.

Ding & Stallone (2001) also utilized isolated rat thoracic aortae to investigate the vasodilatory mechanism of testosterone, and employed methodology very similar to Honda *et al.* (1999). However, Ding & Stallone (2001) demonstrated that neither incubation with glibenclamide nor the K_{Ca} channel blockers TEA and apamin had any effect on the vasodilatory response to testosterone, in vessels preconstricted with noradrenaline (NA). However, incubation with 4-AP did significantly reduce both the potency and efficacy of testosterone (as did preconstriction with KCl), and Ding & Stallone (2001) therefore proposed that testosterone acts *via* a K_V potassium channel opening action. Whilst the study of Ding & Stallone (2001) supports the findings of Yue *et al.* (1995) and has provided further knowledge of the specific potassium channels modulated by testosterone, the results contradict those of Honda *et al.* (1999) using the same preparation, and also other work by the same investigators (Deenadayalu *et al.*, 2001).

Taken together, these four studies provide evidence that testosterone-induced vasodilatation occurs *via* potassium channel opening, having a modulatory effect upon K_V

Table 4 Studies investigating the influence of potassium channel modulation in the vasodilatory mechanism of action of testosterone

Species	*Preparation*	*Contractile agonist*	*Conc^n range of testosterone*	*Incubation*	*Effect*	*Conclusion*	*Reference*
Rabbit	Coronary arteries and thoracic aorta	$PGF_{2\alpha}$ (3 μM)	1–10 μM	None Glibenclamide (3 μM) $BaCl_2$ (3 mM)	95% relaxation ↔ ↓	Dilatation occurs *via* KCa and/or K_V channel opening.	Yue *et al.* (1995)
	1 g isometric tension	KCl (30 mM)		None	42% relaxation		
		calcium + depolarization		Control Verapamil (10 μM) Testosterone (10 μM)	100% contraction ↓↓↓ ↔		
Dog	Conduit and resistance Coronary arteries *in vivo*	None	100 nM–1 μM	None Glibenclamide (10 μM)	86% ↑ in CBF ↓ (resistance vessels only) ↔	Dilatation occurs *via* K_{ATP} channel opening in coronary resistance arteries.	Chou *et al.* (1996)
Rat	Thoracic aortae 0.7 g isometric tension	NA (300 nM)	9–300 μM	None TEA (1 mM) Glibenclamide (3 μM) 4-AP (1 mM)	95% relaxation ↔ ↓↓ ↔	Dilatation occurs *via* K_{ATP} channel opening.	Honda *et al.* (1999)
Rat	Thoracic aortae 2.5 g isometric tension	NA (1 μM)	5–300 μM	None TEA (1 mM) Apamin (0.5 μM) Glibenclamide (10 μM) 4-AP (5 mM)	100% relaxation ↔ ↔ ↔ ↓	Dilatation occurs *via* K_V channel opening.	Ding & Stallone (2001)
		$PGF_{2\alpha}$ (1 μM) NA (1 μM) KCl (80 mM)	50 μM	None None None	89% relaxation 69% relaxation 47% relaxation		
Rat	Mesenteric arterial bed Isobaric tension 15 mmHg	Meth (25 μM)	100 pM–10 μM	None	68% relaxation	Dilatation occurs *via* BK_{Ca} channel opening.	Tep-areenan *et al.* (2002)
		KCl (30 mM) + Meth (25 μM)		None	9%		
		KCl (60 mM)		None	−19%		
		Meth (178 μM)		TBA (300 μM) 4-AP (1 mM)	↓↓↓ ↔		
		Meth (100 μM)		Glibenclamide (10 μM)	↔		
		Meth (178 μM) + 5HT (5 μM)	1 μM	ChTX (100 nM)	↓↓		
Pig	Coronary arteries 2.5 g isometric tension	$PGF_{2\alpha}$ (10 μM)	5–75 μM	None TEA (2 mM) IBTX (20 nM)	97% relaxation ↓↓↓ ↓↓↓	Dilatation occurs *via* BK_{Ca} channel opening.	Deenadayalu *et al.* (2001)
		KCl (80 mM)	5–75 μM	None	5%		

List of abbreviations: 4-AP = 4-aminopyridine; 5-HT = 5-hydroxytrypamine; $BaCl_2$ = barium chloride; CBF = coronary blood flow; ChTX = charybdotoxin; IBTX = iberiotoxin; KCl = potassium chloride; Meth = methoxamine; NA = noradrenaline; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; TBA = tetrabutylammonium; TEA = tetraethylammonium; ↔ = no change in efficacy; ↓ = small reduction in efficacy; ↓↓ = medium reduction in efficacy; ↓↓↓ = large reduction in efficacy.

channels in conduit arteries and upon K_{ATP} channels in resistance vessels. However upon further scrutiny this hypothesis may not hold true.

The conclusions of Yue *et al.* (1995) are based on the observations that the response to testosterone is significantly reduced both by the non-specific potassium channel blocker $BaCl_2$, and under de-polarizing conditions. However, under conditions of high extracellular potassium it is only the *flow* of potassium ions, rather than the channel itself, that is blocked. Since addition of testosterone to the bath has no direct effect upon the trans-membrane potassium gradient, even if it elicited a potassium channel opening action, there would still be a prohibitively large concentration and electrochemical gradient for the potassium ions to overcome, in order to reverse the membrane depolarization responsible for the vasoconstriction. Consequently one would expect the response to testosterone to be abolished under such conditions. Since the vasodilation to testosterone persists (albeit reduced by 50%) in the presence of high extracellular potassium, it is unlikely that a potassium channel opening action is solely responsible for the vasodilatation induced by testosterone in this study.

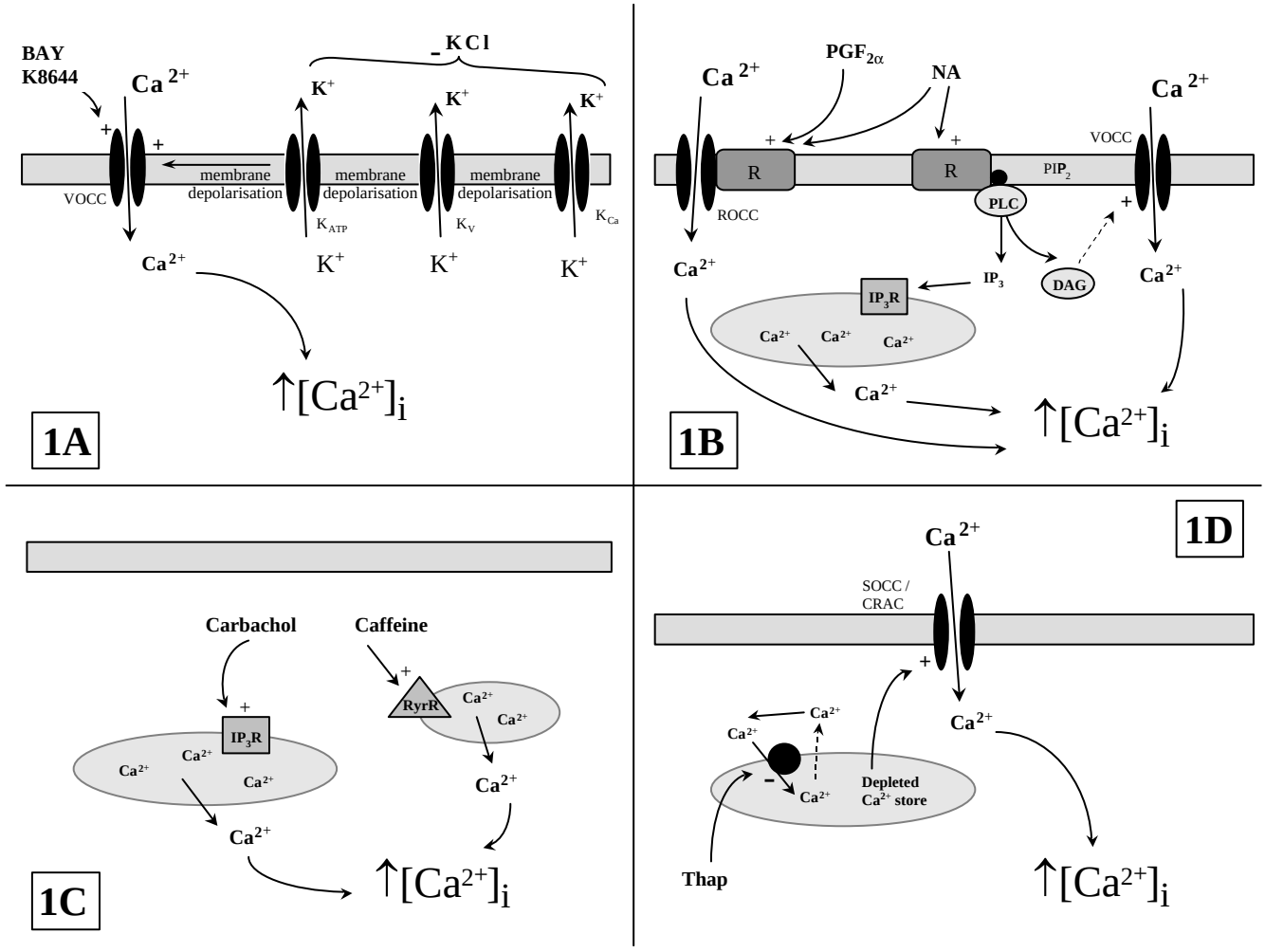

Figure 1 Mechanisms of agonist-induced smooth muscle cell contraction. Smooth muscle contraction is triggered by an elevation in intracellular calcium ($\uparrow[Ca^{2+}]_i$) which catalyses the interaction between the cellular actin and myosin filaments. Under resting conditions an intracellular environment with a high potassium concentration and a low calcium concentration exists with potassium ions moving along their concentration gradient to the extracellular media *via* calcium, voltage and ATP-sensitive potassium channels (K_{Ca}, K_V and K_{ATP}) in the membrane, generating a resting membrane potential of -60 mV. (A) Addition of mM concentrations of extracellular KCl disrupts the potassium concentration gradient, preventing the intracellular to extracellular movement of potassium ions, which are instead retained intracellularly. Depolarization of the membrane potential ensues, activating voltage-operated calcium channels (VOCCs) and triggering extracellular calcium influx (Nelson & Quayle, 1995). BAY K8644 acts as a direct activator of VOCCs (Schramm *et al*., 1983). (B) Noradrenaline (NA) elicits smooth muscle contraction *via* alpha-1 adrenoceptor stimulation, which results in a G-protein coupled activation of phospholipase C (PLC) with subsequent generation of inositol triphosphate (IP_3) and diacyl glycerol (DAG) from the membrane phospholipid phosphatidyl inositol biphosphate (PIP_2). IP_3 acts at its receptor (IP_3R) located on the intracellular membrane of the sarcoplasmic and endoplasmic reticulum, triggering calcium release from these stores whereas DAG activates VOCCs through modulation of chloride channels (Criddle *et al*., 1996). In contrast, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) acts at prostanoid receptors gated directly to receptor-operated calcium channels (ROOCs) (Tosun *et al*., 1997). NA is also reported to activate ROCCs in smooth muscle (Tanaka *et al*., 2000), the proportion of the response attributed to this additional pathway varying between vascular beds. (C) Caffeine and carbachol trigger smooth muscle contraction through stimulation of calcium release from discrete intracellular stores. Carbachol activates the intracellular IP_3 receptors whilst caffeine activates intracellular ryanadine receptors (RyrR) (Xu *et al*., 1994). (D) Thapsigargin (Thap) triggers contraction *via* its inhibitory action on the calcium pumps of the sarcoplasmic and endoplasmic reticulum (SERCA). Under normal conditions a cycling of calcium occurs between these intracellular stores and the cytoplasm, calcium being released from the endoplasmic reticulum and then actively pumped back into these stores *via* the SERCA. In the presence of thapsigargin the SERCA are irreversibly inhibited but the passive calcium release from intracellular stores is unaffected. Consequently an emptying of the intracellular calcium stores ensues, resulting in the activation of store operated calcium channels (SOCCs) and extracellular calcium entry (Treiman *et al*., 1998).

Furthermore the inhibitory effect of $BaCl_2$ is modest even at the high concentrations used.

An alternative explanation of the dilatory action of testosterone may lie with the calcium channels which are activated downstream following exposure to both KCl and $PGF_{2\alpha}$. The high extracellular potassium gradient generated by millimolar KCl triggers membrane de-polarization with subsequent activation of VOCCs (Figure 1A), whilst in contrast $PGF_{2\alpha}$ is proposed to trigger extracellular calcium entry *via* receptor-operated calcium channels (ROCCs) (Figure 1B). The variance in the testosterone-induced dilatation following preconstriction with these agonists, could

be hypothesized to be due to a differing inhibitory efficacy of testosterone upon these calcium channels. The observation that the vasodilatory action of testosterone is compromised in vessels pre-constricted with KCl, suggests that it has less of an inhibitory action upon VOCCs in this preparation. This is supported by the finding that testosterone had no effect upon verapamil-sensitive calcium contractions (Yue *et al*., 1995).

Similarly, in the study of Ding & Stallone (2001), despite showing a reduction in the vasodilatory efficacy of testosterone in vessels pre-constricted with KCl, significant vasodilatation still persisted. In rat thoracic aortae pre-constricted with NA, maximal dilatation to 50 μM testosterone was 69%, which was significantly reduced to 47% in vessels pre-constricted with KCl. However, the efficacy of testosterone could be considered to have three orders of activity, since significantly greater dilatation was seen in vessels preconstricted with $PGF_{2\alpha}$ (89%). Consequently it is an over-simplification of this study to state that the vasodilatory action of testosterone is high when potassium channel function is maintained, and reduced when potassium channel function is compromised. The difference between the activity of testosterone in vessels preconstricted with $PGF_{2\alpha}$ and NA implies a regulatory role upon calcium channel function, and also suggests a preferential antagonism of ROCCs compared to VOCCs – a proportion of the response to NA is attributed to extracellular calcium entry *via* VOCCs, whilst $PGF_{2\alpha}$ relies solely upon ROCC activation (Figure 1B).

However, Tep-areenan *et al*. (2002) have recently provided evidence of a potential potassium channel opening action for testosterone, although the complex methodology utilized in the study makes interpretation of the data somewhat difficult. Tep-areenan *et al*. (2002) studied the vasodilatory action of testosterone in the isolated perfused rat mesenteric bed, at a pressure of 15 mmHg. In order to raise the pressure of the system to near to the *in vivo* level of 100 mmHg, the α_1 adrenoceptor agonist methoxamine was added in conjunction with potassium channel modulators. Due to the varied vasoconstrictive efficacy of these agents, to ensure that all preparations were pressurized to a similar level, a wide variety of concentrations of methoxamine were employed to pre-constrict the system, sometimes in conjunction with additional agonists such as 5-hydroxy tryptamine. However the study of Tep-areenan *et al*. (2002) yields some interesting findings, not in the least that under the conditions described above, testosterone produces vasodilatation within the physiological range (5–50 nM). Tep-areenan *et al*. (2002) also demonstrated that the vasodilatory action of testosterone is markedly attenuated under conditions of high extracellular potassium, whilst in vessels pre-constricted with 60 mM KCl instead of methoxamine, the dilatation is abolished and a vasoconstriction is uncovered. This is indeed consistent with testosterone having a potassium channel opening action as discussed above. Subsequent experiments demonstrated that similar findings were seen in preparations exposed to the K_{Ca} channel blockers tetrabutylammonium (TBA) and charybdotoxin (ChTX), (albeit using a higher concentration of methoxamine in conjunction with 5-HT, and lower concentrations of testosterone), but not the K_V channel blocker 4-AP or the K_{ATP} channel blocker glibenclamide. Tep-areenan *et al*. (2002) therefore conclude that it is the calcium sensitive maxi-K channel (BK_{Ca}) which is activated upon exposure to testosterone.

Deenadayalu *et al*. (2001) have provided more convincing evidence of a similar action of testosterone-induced potassium channel modulation, demonstrating that the vasodilatory efficacy of testosterone is virtually abolished in isolated porcine coronary arteries preconstricted with either 80 mM KCl or the K_{Ca} channel blockers TEA or iberiotoxin (IBTX). These are almost identical findings to those of Tep-areenan *et al*. (2002), but significantly, were reinforced in patch-clamping studies, utilizing individual smooth muscle cells obtained from the primary culture of these vessels. These additional electro-physiological experiments demonstrated that the primary channel regulating the electrical activity of these cells is the BK_{Ca} channel, and that testosterone acted to open this channel. In addition, the cell permeable cyclic guanosine monophosphate (cGMP) analogue, 8-bromo-cGMP, also increased BK_{Ca} channel activity, and testosterone increased the cellular production of cGMP. These findings provide convincing evidence that testosterone-induced vasodilatation occurs *via* the opening of BK_{Ca} channels in this preparation, and have suggested a potential effector mechanism in the production of cGMP.

Testosterone as a calcium channel antagonist

Of the studies discussed above only those of Deenadayalu *et al*. (2001) and Tep-areenan *et al*. (2002) report the vasodilatory action of testosterone to be abolished under conditions of high extracellular potassium, consistent with the hypothesis of testosterone triggering vasodilatation *via* a potassium channel-opening action. The studies of Yue *et al*. (1995) and Ding & Stallone (2001) could be interpreted to be supportive of a calcium antagonistic action for testosterone, and numerous studies provide more direct evidence for such an action (Table 5).

The first paper to propose a calcium antagonistic action for testosterone was that of Perusquía & Villalón (1999) who studied the vasodilatory action of 5β-dihydrotestosterone (5β-DHT) in endothelial-denuded isolated rat thoracic aortae. Perusquía & Villalón (1999) demonstrated a vasodilatory action in vessels preconstricted with KCl or NA, which was greater in KCl-preconstricted vessels.

Since 5β-DHT completely reversed the preconstriction induced by the VOCC agonist BAY K8644, Perusquía & Villalón (1999) concluded that 5β-DHT was acting to inhibit both ROCCs and VOCCs, but had a higher efficacy upon VOCCs. Consistent with this hypothesis were the subsequent observations that 5β-DHT was able to abolish calcium-induced contractions under depolarizing conditions mediated *via* VOCCs, and shared a similar efficacy as the VOCC blocker nifedipine in inhibiting contractions to KCl and NA (Perusquía & Villalón, 1999).

An elegant series of experiments by Raouf Khahil's group at the University of Mississippi has also provided evidence for a calcium antagonistic action of testosterone. In initial experiments Crews & Khalil (1999a) demonstrated that testosterone dilated male porcine coronary arteries preconstricted with $PGF_{2\alpha}$ or KCl, but had no inhibitory action upon caffeine-induced contractions which occur *via* the release of calcium from ryanadine sensitive intracellular stores (Figure 1C). Testosterone also inhibited $PGF_{2\alpha}$ or KCl-induced entry of $^{45}Ca^{2+}$ into de-endothelialized porcine

Table 5 Studies investigating the influence of calcium channel modulation in the vasodilatory mechanism of action of testosterone

Species	*Preparation*	*Contractile agonist*	*Conc^n range of testosterone*	*Incubation*	*Effect*	*Conclusion*	*Reference*
Rat	Thoracic aortae (endothelial denuded) 1 g isometric tension	NA (0.3 μM)	7.5–120 μM	Endothelial denudation	50% relaxation	Dilatation occurs *via* VOCC blockade with some efficacy at ROCCs.	Perusquía & Villalón (1999)
		KCl (60 mM)	(5β-DHT)	Endothelial denudation	75% relaxation		
		KCl (60 mM) + BAY (10 μM)		Endothelial denudation	100% relaxation		
		KCl (60 mM)	/	Vehicle	100% contraction		
				Nifedipine (90 nM)	↓↓↓		
				5β-DHT (150 μM)	↓		
		NA (0.3 μM)	/	Vehicle	100% contraction		
				Nifedipine (90 nM)	↓		
				5β-DHT (150 μM)	↓		
		depolarization + $CaCl_2$ (1.5 mM)	/	Vehicle	100% contraction		
				5β-DHT (150 μM)	↓↓↓		
Pig	Coronary arteries (endothelial denuded)	$PGF_{2\alpha}$ (10 μM)	0.1–100 μM	Endothelial denudation	90% relaxation	Dilatation occurs *via* VOCC/ROCC blockade.	Crews & Khalil (1999a)
		KCl (96 mM)		Endothelial denudation	75% relaxation		
		Caff (2.5 mM)	/	Vehicle	100% contraction		
	2 g isometric tension			Testosterone (10 μM)	↔		
Rat	Thoracic aortic strips (endothelial denuded)	NA (10 μM)	0.1–10 μM	Endothelial denudation	90% relaxation	Dilatation occurs *via* VOCC/ROCC blockade.	Crews & Khalil (1999b)
		KCl (96 mM)		Endothelial denudation	70% relaxation		
		Caff (2.5 mM)	/	Vehicle	100% contraction		
	2 g isometric tension			Testosterone (10 μM)	↔		
Pig	Coronary artery smooth muscle cells	$PGF_{2\alpha}$ (10 μM)	/	Vehicle	100% contraction + ↑ $[Ca^{2+}]_i$	Dilatation occurs *via* VOCC/ROCC blockade.	Murphy & Khalil (1999)
				Testosterone (0.1 μM)	↓↓		
		KCl (51 mM)	/	Vehicle	100% contraction + ↑ $[Ca^{2+}]_i$		
				Testosterone (0.1 μM)	↓		
		Caff (10 mM)	/	Vehicle	100% contraction + ↑ $[Ca^{2+}]_i$		
				Testosterone (0.1 μM)	↔		
		Carb (10 μM)	/	Vehicle	100% contraction + ↑ $[Ca^{2+}]_i$		
				Testosterone (0.1 μM)	↔		
		$PGF_{2\alpha}$ (10 μM)	10–100 nM	None	60% ↓ $[Ca^{2+}]_i$		
		KCl (51 mM)		None	75% ↓ $[Ca^{2+}]_i$		
Rat	Coronary arteries	$PGF_{2\alpha}$ (100 μM)	1–1000 μM	None	80% relaxation	Dilatation occurs *via* VOCC/ROCC blockade with some activity upon PKC	English *et al.* (2002)
		KCl (100 mM)		None	100% relaxation		
	Isometric tension equivalent to 100 mmHg	$PDBu^{-Ca}$ (1 μM)		None	40% relaxation		
Rat	Pulmonary arteries	$PGF_{2\alpha}$ (100 μM)	1–1000 μM	None	30% relaxation	Dilatation occurs *via* VOCC/ROCC blockade with some activity upon PKC	Jones *et al.* (2002a)
		KCl (80 mM)		None	30% relaxation		
	Isometric tension equivalent to 17.5 mmHg	BAY (1 μM)		None	30% relaxation		
		$PDBu^{-Ca}$ (1 μM)		None	10% relaxation		
		Thap (1 μM)		None	2% relaxation		

List of abbreviations: BAY = BAY K8644; Caff = caffeine; Carb = carbachol; DHT = dihydrotestosterone; KCl = potassium chloride; NA = noradrenaline; $PDBu^{-Ca}$ = phorbol dibutyrate in calcium free saline; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; PKC = protein kinase C; ROCC = receptor operated calcium channel; Thap = thapsigargin; VOCC = voltage operated calcium channel; ↔ = no change in efficacy; ↓ = small reduction in efficacy; ↓↓ = medium reduction in efficacy; ↓↓↓ = large reduction in efficacy.

coronary artery strips (Crews & Khalil, 1999a). Identical findings were found in additional experiments utilizing de-endothelialized isolated rat thoracic aortic strips (Crews & Khalil, 1999b). Again testosterone dilated vessels pre-constricted with NA or KCl, but had no inhibitory action upon caffeine-induced contractions (Crews & Khalil, 1999b). These studies demonstrate that the vasodilatory efficacy of testosterone is restricted to conditions where extracellular calcium entry occurs, implying blockade of ROCC and VOCCs. In subsequent work Murphy & Khalil (1999) showed a similar mechanism of action of testosterone in isolated porcine coronary artery smooth muscle cells.

Testosterone pre-treatment significantly inhibited the smooth muscle cell contraction and reduced the increase in intracellular calcium $[Ca^{2+}]_i$ associated with exposure to $PGF_{2\alpha}$ or KCl but not caffeine or carbachol (an activator of IP_3-mediated intracellular calcium release) (Figure 1C) (Murphy & Khalil, 1999). These studies provide convincing evidence that testosterone has little effect upon either calcium or IP_3-mediated release of calcium from intracellular stores. The observation that testosterone caused marked attenuation of responses induced by KCl provides strong evidence that testosterone has an antagonistic action upon VOCCs. However, in all three papers the inhibitory efficacy and potency of testosterone was greater against responses to $PGF_{2\alpha}$ or NA, which suggests that testosterone must also be efficacious in blocking ROCCs and may indeed have a higher affinity for this type of calcium channel.

Data recently published by our group are also supportive of a calcium antagonistic mechanism of action of testosterone. Utilizing isolated rat coronary arteries we have demonstrated that testosterone elicits marked vasodilatation in vessels preconstricted with $PGF_{2\alpha}$, or KCl (English *et al*., 2002). The vasodilatory action of testosterone was significantly attenuated in vessels pre-constricted *via* phorbol dibutyrate (PDBu) in calcium free saline, conditions under which only intracellular, non-calcium dependent contractile pathways are activated (English *et al*., 2002). Similar findings were subsequently found in isolated pulmonary arteries (Jones *et al*., 2002a), although as previously demonstrated, the efficacy of testosterone was significantly lower in this vascular bed (English *et al*., 2001). Vasodilation to testosterone was split into three orders of magnitude, dependent upon the mechanism of action of the pre-contractile agent (Jones *et al*., 2002a). Similar testosterone-induced dilatation occurred in vessels pre-constricted *via* either $PGF_{2\alpha}$, KCl or the VOCC agonist BAY K8644, with significantly lower responses being observed in vessels pre-constricted with PDBu in calcium free saline (Jones *et al*., 2002a). The response was abolished in vessels pre-constricted with thapsigargin which actives store-operated calcium channels (SOCCs) following depletion of the intercellular calcium stores *via* the inhibition of the calcium transporter located in the membrane of the sarcoplasmic reticulum (Figure 1D) (Jones *et al*., 2002a). Again these studies are supportive of a calcium channel antagonistic action upon VOCCs and ROCCs.

Taken together these studies demonstrate the reliance of testosterone-mediated vasodilatation upon specific mechanisms of calcium signalling. Extracellular calcium entry would appear to be a pre-requisite for a vasodilatory action since testosterone has little inhibitory activity upon contractile responses generated through the release of calcium from intracellular stores. Furthermore, the type of channel through which extracellular calcium enters the cell is also a contributory factor. Testosterone elicits marked vasodilatation is vessels pre-constricted *via* agonists which activate ROCCs and VOCCs, but not SOCCs.

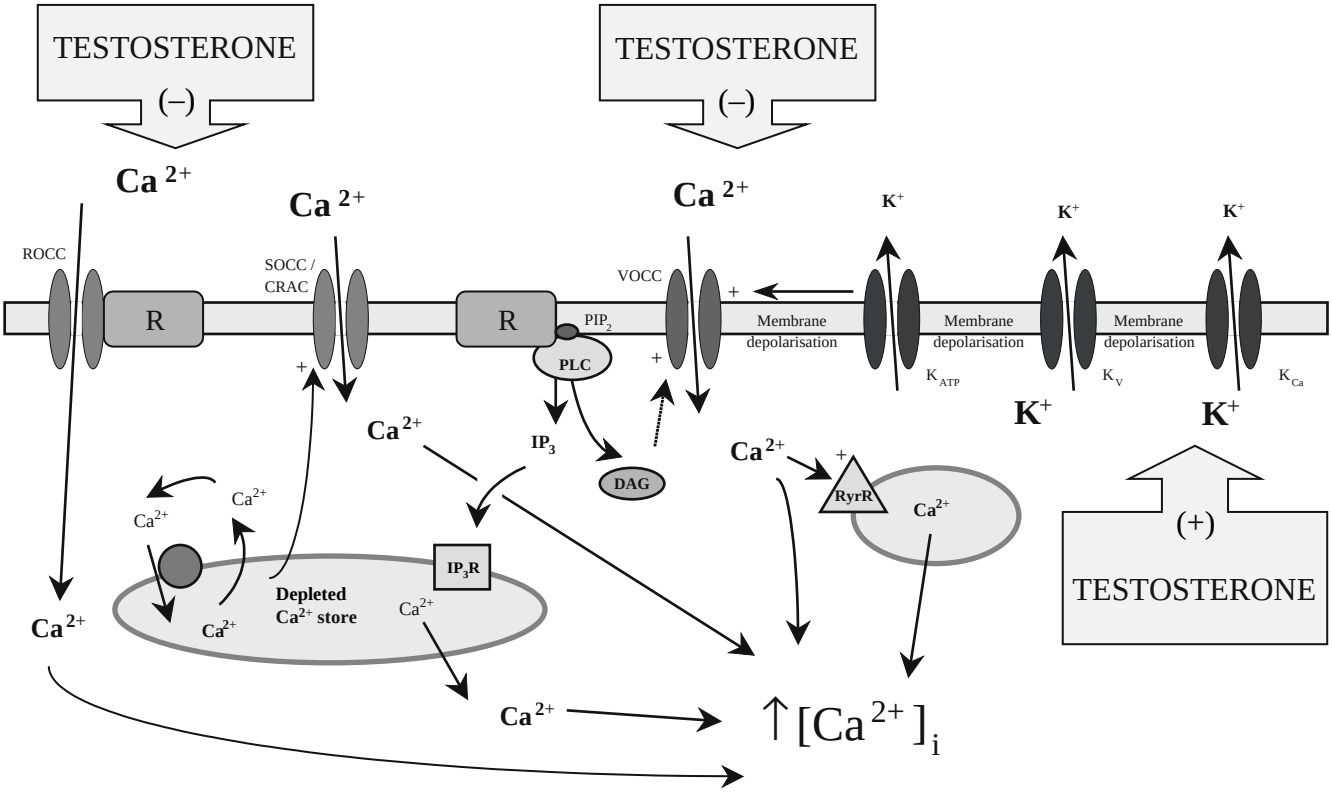

Figure 2 Figure summarizing the proposed sites of action of testosterone which underlie its vasodilatory activity. List of abbreviations: $\uparrow[Ca^{2+}]_i$ = elevation in intracellular calcium; DAG = diacyl glycerol; IP_3 = inositol triphosphate; IP_3R = inositol triphosphate receptor; K_{ATP} = ATP-sensitive potassium channel; K_{Ca} = calcium-sensitive potassium channel; KCl = potassium chloride; K_V = voltage-sensitive potassium channel; NA = noradrenaline; PLC = phospholipase C; $PGF_{2\alpha}$ = prostaglandin $F_{2\alpha}$; PIP_2 = phosphatidyl inositol biphosphate; ROOC = receptor-operated calcium channel; RyrR = ryanadine receptor; SOCC = store operated calcium channel; Thap = thapsigargin; VOCC = voltage-operated calcium channel.

Conclusion

Clearly the majority of studies conducted into determining the dilatory mechanism of action of testosterone can be interpreted as being supportive of a calcium antagonistic action, since the efficacy of testosterone is clearly linked to the mechanism of action of the precontractile agonist. The specific channels upon which testosterone is proposed to act and the studies which support each action are shown in Figure 2.

The observation that little (Crews & Khalil, 1999a, b; Murphy & Khalil, 1999; Ding & Stallone, 2001) or no (Perusquía & Villalón, 1999; English *et al*., 2002; Jones *et al*., 2002a) reduction in the efficacy of testosterone is seen in vessels pre-constricted with KCl, demonstrate that testosterone cannot be acting to open potassium channels as has been previously suggested. Only the studies of Deenadayalu *et al*. (2001) and Tep-areenan *et al*. (2002) report an abolition of the response to testosterone under such conditions, consistent with a potassium channel opening action. However, the findings of the report of Tep-areenan *et al*. (2002) are difficult to interpret due to the inconsistent experimental conditions utilized. Therefore only the study of Deenadayalu *et al*. (2001) convincingly demonstrate that testosterone-mediated vasodilatation occurs *via* activation of potassium channels, data which are supported by the presence of electrophysiological studies and proposition of an intermediate signalling molecule.

Surprisingly few studies have utilized patch clamp methodology to examine the calcium antagonistic action of testosterone, and the absence of such studies precludes the confirmation of this mechanism of action. Only one paper, as an aside to studying the vasodilatory mechanism of action of the female sex hormone 17β oestradiol, has looked for an inhibitory action of testosterone upon calcium channel function: Nakajima *et al*. (1995) report that whilst 17β oestradiol attenuates calcium flux occurring *via* VOCCs in an embryonic rat aortic smooth muscle cell line, testosterone did not share this activity. However, until data is obtained from primary vascular smooth muscle cells, obtained from adult or at least post-natal tissue, such conclusions must be drawn with caution.

The discrepancy between the studies discussed in this review in proposing a vasodilatory mechanism for testosterone is not apparent, but the variance in the species and vascular preparations used is likely to be contributory. Taking this into consideration, the lack of mechanistic studies conducted in human preparations is likely to be significant, and clearly this is a major gap in the current literature. Such data, especially from cellular patch-clamp studies, are essential if the exact mechanism of action of testosterone is to be unearthed, and the promising results from the preliminary clinical studies of testosterone therapy in male patients with CAD are to be fully realised.

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