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RESEARCH PAPER

Effects of T-type calcium channel blockers and thalidomide on contractions of rat vas deferens

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Background and purpose: In rat vas deferens, nerve mediated-contractions to a single electrical stimulus consist of an early purinergic and a later adrenergic component with differing sensitivities to L-type calcium channel blockers. We have investigated the effects of the T-type calcium channel blockers mibefradil and (1S, 2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl cyclopropanecarboxylic dihydrochloride (NNC 55-0396) against contractions in rat vas deferens. In addition, the actions of thalidomide were examined.
Experimental approach: Prostatic and epididymal portions of rat vas deferens were stimulated with a single electrical stimulus every 5 min, and mouse whole vas deferens was stimulated with 40 pulses at 10 Hz every 5 min.
Key results: Both mibefradil and NNC 55-0396 (100 μM) produced inhibition of contractions of epididymal portions (42 ± 13%, $n = 7$, and 43 ± 4%, $n = 15$, of control respectively). However, both agents produced small inhibitions of responses in prostatic portions, presumably by L-type calcium channel block. Thalidomide (100 μM) inhibited contractions in epididymal (55 ± 4% of control, $n = 17$) but not in prostatic portions of rat vas deferens. Thalidomide (10–100 μM) also inhibited contractions in mouse vas deferens.
Conclusions and implications: The T-type calcium channel blockers mibefradil and NNC 55-0396 block particularly the adrenoceptor-mediated, nifedipine-resistant response to nerve stimulation in rat vas deferens, and this may suggest that this component involves T-type calcium channels. In addition, thalidomide has actions that resemble those of the T-type calcium channel blockers, in that it blocks nifedipine-resistant contractions in epididymal portions.
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Keywords: NNC 55-0396; mibefradil; thalidomide; rat vas deferens; mouse vas deferens; T-type calcium channels; α_1-adrenoceptor

Abbreviations: IBMX, isobutylmethylxanthine; NNC 55-0396, (1S, 2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl cyclopropanecarboxylic dihydrochloride; SB203580, 4-[5-(4-fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine

Introduction

In rat vas deferens, nerve mediated-contractions to a single electrical stimulus consist of an early purinergic and a later adrenergic component with differing sensitivities to L-type calcium channel blockers. In the prostatic portion, contractions to a single stimulus are largely purinergic, and this response is abolished by L-type calcium channel blockers such as nifedipine (Blakeley *et al.*, 1981; French and Scott, 1981; Brown *et al.*, 1983). In the epididymal portion, contractions to a single stimulus are largely adrenergic, and this response is unaffected by nifedipine. These results have been interpreted as meaning that the early purinergic component of the nerve-evoked contraction involves calcium entry through L-type calcium channels, and the later adrenergic component involves calcium stores. However, a more careful conclusion would be that the later component does not involve L-type channels.

T-type calcium channel blockers have been available for a number of years, but functional studies in vas deferens have not been carried out until recently. Shishido *et al.* (2009) have shown that a component of contractions to trains of pulses in guinea-pig vas deferens is susceptible to T-type calcium channel blockers. In that study, the contractile response to trains of pulses at 40 Hz was biphasic with a peak reached after about 1 s, consisting of purinergic and adrenergic components, with a late contraction after about 5 s due

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probably to the overflow of transmitter from synaptic sites. This late component was adrenergic. The late but not the early adrenergic component was blocked by the L-type blocker nifedipine, and the early component was blocked by the T-type blocker mibefradil. This led us to examine whether the adrenergic (nifedipine-resistant) component to a single electrical stimulus in rat vas deferens is sensitive to T-type calcium channel blockers. In the rat vas deferens, the adrenergic component of the response to nerve stimulation can be isolated, anatomically and pharmacologically, to the epididymal portion in the presence of nifedipine.

We have investigated the effects of the T-type calcium channel blockers mibefradil and (1S, 2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl cyclopropanecarboxylic dihydrochloride (NNC 55-0396) against contractions to single pulse nerve stimulation in rat vas deferens. In addition, we have investigated the actions of thalidomide on the contractile responses of rat vas deferens, as we have found from other ongoing studies that thalidomide produces smooth muscle relaxation (Seto *et al.*, 2009). Thalidomide interferes with the effects of tumour necrosis factor-α (TNF-α) (Moreira *et al.*, 1993) and has immuno-modulatory and anti-angiogenic actions (Bauer *et al.*, 1998), but no direct smooth muscle relaxant actions have been reported.

We found that the T-type calcium channel blockers mibefradil and NNC 55-0396 more potently inhibited contractions of epididymal than prostatic portions, and thalidomide only inhibited contractions in epididymal portions of rat vas deferens. Effects of thalidomide did not involve glibenclamide-sensitive potassium channels, p38 mitogen-activated protein kinase (MAP kinase) or phosphodiesterase.

Some of these results have been published in abstract form (Seto and Docherty, 2009).

Methods

All studies conform to the Declaration of Helsinki and have been approved by the Department of Health and by the RCSI Research Ethics Committee. Male Wistar rats (250–350 g) and male C57BL6J mice (20–25 g) were obtained from Harlan Laboratories (Bicester, UK) and killed by CO_2 overdose. Epididymal and prostatic portions of rat vas deferens and whole mouse vas deferens were set up in organ baths under 1 g (rat) or 0.5 g (mouse) resting tension in Krebs-Henseleit solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, glucose 11.1, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.0, EDTA 0.03 and ascorbic acid 0.28. Bathing fluid was changed every 15 min except during contractile studies.

Rat vas deferens

In experiments investigating the ability of test agents to inhibit the isometric twitch in prostatic and epididymal portions of vas deferens, tissues were placed between platinum electrodes and stimulated every 5 min with a single stimulus (0.5 ms pulses, supramaximal voltage) to produce isometric contractions. Nifedipine (10 μM) was present in all studies in epididymal portions to block the purinergic component of the twitch. Test agents or vehicle were added cumulatively in 1 log unit increments at 5 min intervals. An isometric twitch was obtained following 5 min exposure to each test drug concentration or following exposure to the vehicle. For the final concentration of antagonist, the response was measured 15 min after administration. Equivalent additions of vehicle produced only small effects on the stimulation-evoked contraction. In interaction studies, the phosphodiesterase inhibitor, isobutyl-methylxanthine (IBMX; 100 μM), the K_{ATP} channel blocker glibenclamide (10 μM), the p38 MAP kinase inhibitor, 4-[5-(4-fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine (SB203580; 3 μM) or vehicle was added 30 min prior to thalidomide. In some experiments, rather than examine a range of concentrations, a single concentration (100 μM) of thalidomide or other agents was examined following vehicle or other test agent. Hence, results shown in Figures 5 and 6 have mostly been obtained from more experiments than those of Figures 3 and 4. In some studies, thalidomide (100 μM) or vehicle was added 15 min after administration of NNC 55-0396 (100 μM).

Mouse vas deferens

Following an equilibration period, tissues were stimulated at a frequency of 10 Hz for 4 s every 5 min. When consistent responses had been obtained, a cumulative concentration response curve to thalidomide (1–100 μM), or equivalent amounts of vehicle, were obtained. Drug or vehicle was added immediately after a stimulation period, and the next stimulation occurred 5 min later in the presence of thalidomide or vehicle.

Statistics

Values are mean ± s.e. mean from *n* experiments. Effects of test agents on nerve stimulation-evoked responses were compared with the effects of vehicle by analysis of variance and Tukey's or Dunnett's post-test. Statistical and graphical analysis was carried out using Instat and GraphPad Prism for MacIntosh.

Materials

Isobutyl-methylxanthine (IBMX), glibenclamide and nifedipine were from Sigma (Dublin, Ireland); NNC 55-0396 ((1S, 2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl cyclopropanecarboxylic dihydrocholide), SB203580 (4-[5-(4-fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine), thalidomide and mibefradril were obtained from Tocris (Bristol, UK). Drugs were dissolved in distilled water, except for nifedipine (100% ethanol: maximum bath concentration, 0.1%) and thalidomide (100% dimethylsulphoxide: maximum final bath concentration 0.2%). Drugs were diluted in distilled water. Drug/molecular target nomenclature conforms to the BJP's Guide to Receptors and Channels (Alexander *et al.*, 2008).

Results

Epididymal and prostatic portions of rat vas deferens

Figure 1 shows typical responses of the two portions of the rat vas deferens: a monophasic response in prostatic portions that is largely purinergically mediated and is nifedipine-sensitive and a biphasic response in epididymal portions with a late α_1-adrenoceptor-mediated component resistant to nifedipine (Figure 1). The early purinergic response occurs with a peak at approximately 0.30 s, and the second adrenergic responses occur with a peak at approximately 0.65 s, post stimulus (Figure 1). In the presence of nifedipine, the early purinergic component is absent in epididymal portions, leaving only the later adrenergic component (Figure 2).

In prostatic portions of rat vas deferens, the isometric twitch to a single stimulus was monophasic with a maximum of 1.04 ± 0.0.10g (n = 18) (see Figure 1). Nifedipine (10 μM) abolishes this purinergic contraction (data not shown). Mibefradil and NNC 55-0396 (100 μM) both significantly reduced the stimulation-evoked contractions in prostatic portions (Figure 3).

In epididymal portions in the presence of nifedipine, single pulse stimulation produced a contraction of 1.09 ± 0.09g (n = 19). In these tissues, over a range of concentrations, both mibefradil and NNC 55-0396 (100 μM) significantly reduced the response to a single stimulus (n = 4 each; Figure 3), and both produced greater inhibition than in prostatic portions (Figure 3). When all results obtained with mibefradil or NNC 55-0396 (100 μM) are included, the epididymal response was reduced to about 40% of control (Figure 4).

Thalidomide (10–100 μM) significantly reduced contractions in epididymal portions but not in prostatic portions (Figure 5). Glibenclamide (10 μM) did not significantly affect contractions of prostatic or epididymal portions, and following glibenclamide, thalidomide still produced inhibition of contractions in epididymal portions (see Figures 4 and 5).

IBMX (100 μM) significantly reduced contractions in both epididymal and prostatic portions, producing greater inhibition in prostatic (reduced to 17 ± 2% of control, n = 5) than epididymal portions (reduced to 42 ± 8% of control, n = 8) (see Figure 4). However, when given subsequent to IBMX (100 μM), thalidomide (100 μM) reduced the contractile response still further, in epididymal portions (Figure 4). Vehicle post IBMX did not significantly alter the response to IBMX (38 ± 5% of control, n = 4).

The p38 MAP kinase inhibitor SB203580 (3 μM) had no effect in epididymal portions (98 ± 4%, n = 4) but produced a small decrease in the response to a single stimulus in prostatic portions (87 ± 9% of control), although this did not reach significance. SB203580 (10 μM) produced no further effect in prostatic portions. SB203580 (3 μM) did not affect the inhibitory response to thalidomide (100 μM) (see Figure 4).

Thalidomide or NNC 55-0396 (both 100 μM) significantly reduced contractions to a single stimulus, but as both were

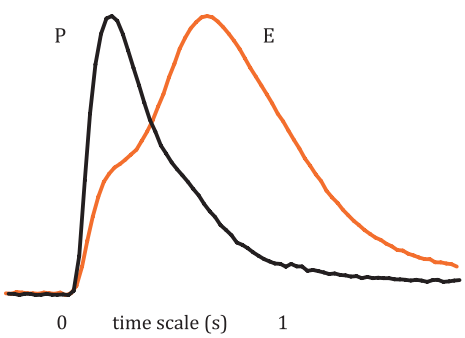

Figure 1 Recordings of the contraction to a single electrical stimulus in prostatic (P, monophasic) and epididymal (E, biphasic) portions of rat vas deferens. The vertical axis shows tension that has been autoscaled so that the response is of the same magnitude in both portions. Mean values for tension obtained in each portion of vas deferens are shown in the results section. The time scale shows 1 s (the tick marks on the abscissa are 0.5 s per unit.), and time 0 indicates the electrical stimulus. The first phase of the response occurs at about 0.30 s, and the second phase occurs at about 0.65 s.

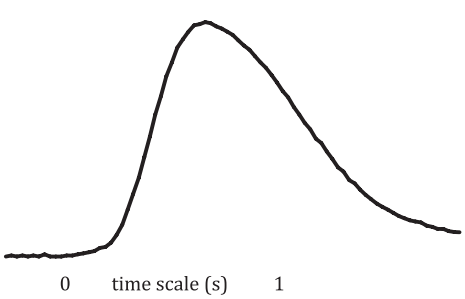

Figure 2 Recordings of the contraction to a single electrical stimulus in an epididymal (E) portion of rat vas deferens in the presence of nifedipine (10 μM). Note the absence of an early component to the response. The time scale shows 1 s (the tick marks on the abscissa are 0.5 s per unit.), and time 0 indicates the electrical stimulus.

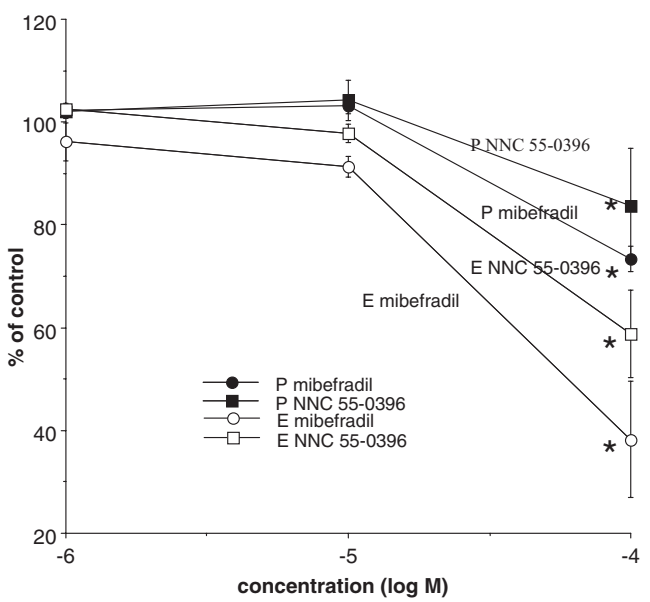

Figure 3 Concentration-response curves for the inhibition by NNC 55-0396 and mibefradil of the isometric contraction to a single stimulus in epididymal (E) and prostatic (P) portions of rat vas deferens. Responses in the presence of test drug are expressed as a percentage of the control response. Vertical bars indicate s.e. of mean from four to eight experiments. *P < 0.05, significantly different from the effects of vehicle. NNC 55-0396, (1S, 2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl cyclopropanecarboxylic dihydrochloride.

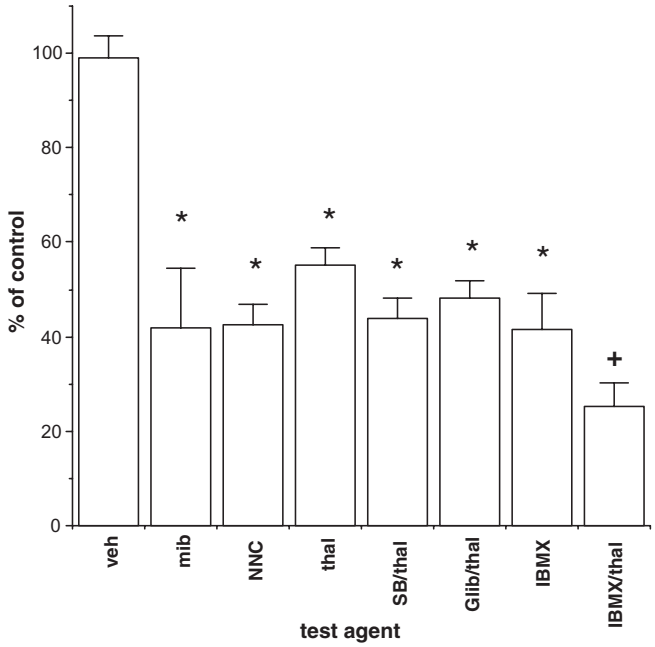

Figure 4 Effects of mibefradil (mib), NNC 55-0396 (NNC), IBMX or thalidomide (all 100 μM) on the isometric contraction to a single electrical stimulus in epididymal portions of rat vas deferens in the presence of nifedipine (10 μM). Also shown are the effects of thalidomide in the presence of glibenclamide (10 μM), IBMX (100 μM) or the p38 MAP kinase inhibitor SB203580 (3 μM), expressed as inhibition of the response post test agent (glibenclamide data taken from Figure 5). IBMX significantly reduced the contraction to a single stimulus, but the other agents (glibenclamide, SB203580) had no effect (see text). Vertical bars indicate s.e. of mean from 5 to 17 experiments. *$P < 0.05$, significantly different from the effects of vehicle; +$P < 0.05$, significantly different from the response to IBMX alone. IBMX, isobutylmethylxanthine; NNC 55-0396, (1S, 2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl cyclopropanecarboxylic dihydrochloride; SB203580, 4-[5-(4-fluorophenyl)-2-[4-(methylsulfonyl)phenyl]-1H-imidazol-4-yl]pyridine.

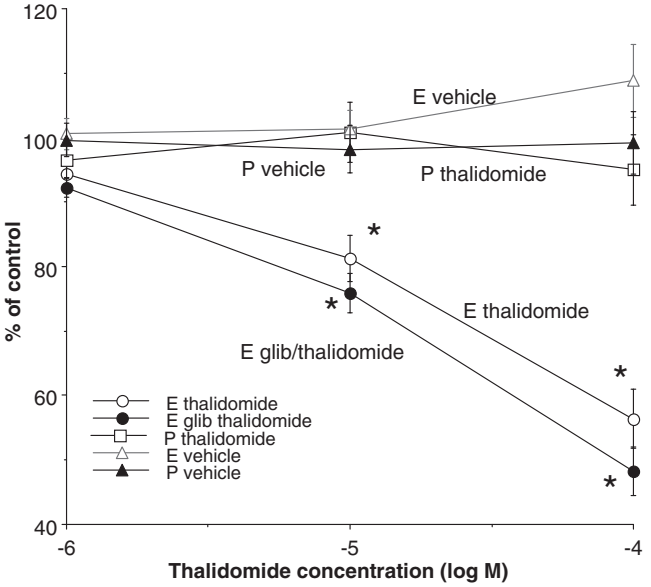

Figure 5 Concentration-response curves for the inhibition by thalidomide of the isometric contraction to a single stimulus in epididymal (E) and prostatic (P) portions of rat vas deferens and the effect of pre-exposure to glibenclamide (10 μM) (glib). Responses in the presence of thalidomide are expressed as a percentage of the control response. Glibenclamide did not significantly alter the response to thalidomide. Also shown are the effects of cumulative addition of vehicle, replacing thalidomide (dashed lines). Vertical bars indicate s.e. of mean from four to eight experiments. *$P < 0.05$, significantly different from the effects of vehicle.

approximately equipotent, the combination produced further inhibition compared with the effect of the combination of NNC 55-0396 and vehicle (Figure 6). This combination (thalidomide + NNC 55-0396) did not abolish responses, but all responses were abolished by the further addition of the α_2-adrenoceptor agonist xylazine (1 μM; Figure 6), confirming that the residual response following NNC 55-0396 and thalidomide was a nerve-mediated response.

In mouse vas deferens, stimulation with 40 pulses at 10 Hz (in the absence of nifedipine) produced a contraction of 0.64 ± 0.16 g (n = 5). Thalidomide (10 and 100 μM) produced significant inhibitions of the contraction (Figure 7).

Discussion

In this study, we have examined the effects of T-type calcium channel blockers on the biphasic response of rat vas deferens to single pulse electrical stimulation. In addition, we have investigated the effects of thalidomide.

In rat whole vas deferens, the electrical stimulation-evoked contraction to a single stimulus consists of a biphasic response, the first phase of which occurs at approximately 0.3 s post stimulus and is purinergic and predominates in prostatic portions, and the second phase of which occurs approximately 0.65 s post stimulus and is α_1-adrenoceptor mediated and predominates in the epididymal portion (see Brown *et al.*, 1983 and Figures 1 and 2). The second adrenergic phase can be examined in isolation in the epididymal portion in the presence of nifedipine which eliminates the first non-adrenergic response. Nifedipine (10 μM) abolishes the first purinergic response in prostatic portions of vas deferens.

Both mibefradil and NNC 55-0396 were more effective at inhibiting contractions of epididymal portions than prostatic portions, in marked contrast to nifedipine. However, both did significantly reduce contractions to nerve stimulation in prostatic portions. These results suggest that these drugs have very limited selectivity for T-type channels (assuming that their effects in the epididymal portion indeed involve T-type channels) over L-type channels, at least in this model system, and it is perhaps fortunate that L- and T-type responses are so clearly separable in the rat vas deferens.

In this study we also examined the actions of thalidomide in the rat vas deferens because we had evidence from other ongoing studies that it produces smooth muscle relaxation (see Seto *et al.*, 2009). Thalidomide interferes with the effects of TNF-α (Moreira *et al.*, 1993) and has immuno-modulatory and anti-angiogenic actions (Bauer *et al.*, 1998). It is effective in the treatment of skins disorders due to leprosy (see Walker *et al.*, 2007) and is being investigated as an anticancer drug (Varker *et al.*, 2008) and in the treatment of HIV infection (see Joglekar and Levin, 2004).

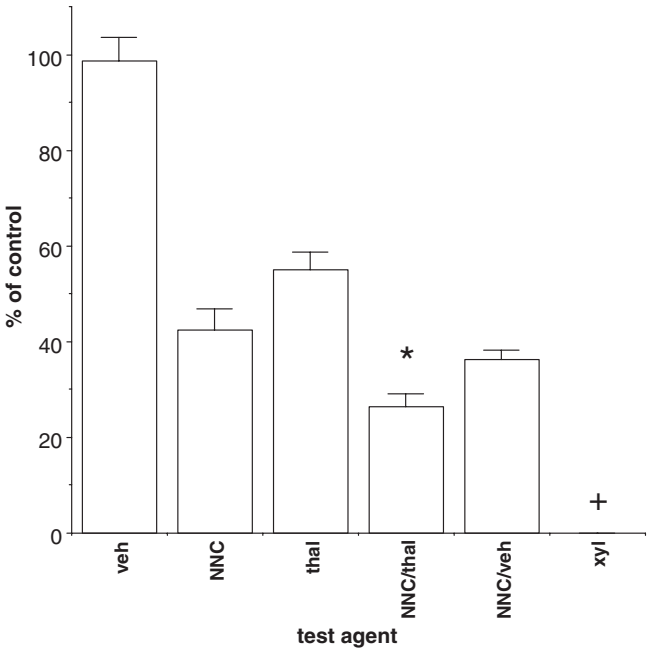

Figure 6 Effects of NNC 55-0396 (NNC) or thalidomide (both 100 μM) and the interaction between NNC 55-0396 and thalidomide or vehicle on the isometric contraction to a single electrical stimulus in epididymal portions of rat vas deferens in the presence of nifedipine (10 μM). Vertical bars indicate s.e. of mean from 4–17 experiments. Also shown are the effects of the α_2-adrenoceptor agonist, xylazine given after thalidomide or NNC 55-0396, or their combination. The effects of all drug and drug combinations were significantly different from the effects of vehicle. In the case of combinations, the combined inhibitory effect is shown. Vertical bars indicate s.e. of mean from 6 to 17 experiments. *$P < 0.05$, significantly different from the effects of NNC 55-0396 followed by thalidomide and NNC 55-0396 followed by vehicle; +$P < 0.05$, responses following xylazine were abolished ($P < 0.05$). NNC 55-0396, (1S, 2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl cyclopropanecarboxylic dihydrochloride.

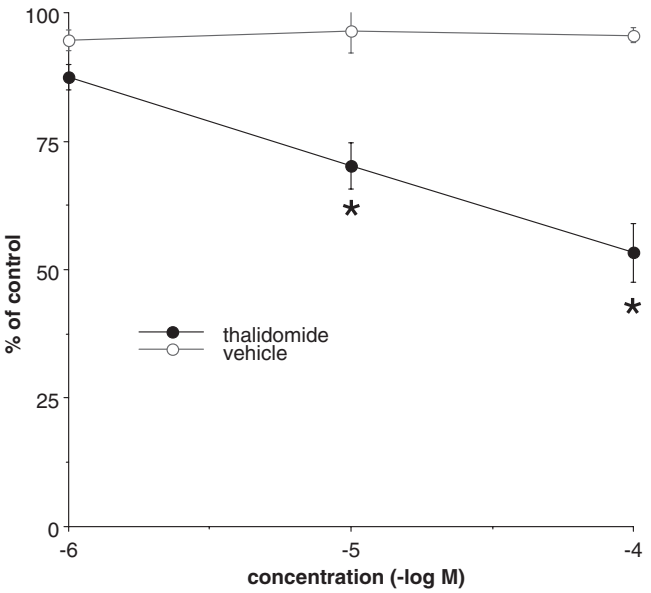

Figure 7 Effects of thalidomide on the isometric contraction to electrical stimulation with 40 pulses at 10 Hz in mouse whole vas deferens. Vertical bars indicate s.e. of mean from five experiments. *$P < 0.05$, significantly different from the effects of vehicle.

Thalidomide (100 μM) significantly inhibited contractions to single pulse stimulation in epididymal portions, but not prostatic portions, of rat vas deferens. These results confirm that thalidomide is not an L-type calcium channel blocker but behaves similarly to T-type channel blockers, at least in rat vas deferens. We have confirmed that thalidomide is not an α_1-adrenoceptor antagonist in ligand binding and functional studies (see Seto *et al.*, 2009), so that the action is not as a receptor antagonist of the α_1-adrenoceptor-mediated contraction of epididymal portions. Inhibitors of phosphodiesterase such as IBMX reduce stimulation-evoked contractions by increasing cAMP levels in the guinea-pig vas deferens (Stjärne *et al.*, 1979), and we have found a similar inhibition of contractions by IBMX (100 μM) in rat vas deferens. However, unlike thalidomide, IBMX inhibits responses in both portions of the rat vas deferens and indeed produces greater inhibition in prostatic than epididymal portions. However, in epididymal portions, in the presence of IBMX, thalidomide produced further inhibition. Glibenclamide (10 μM), by blocking K_{ATP} ($K_{IR}.6x$) channels, did not influence the inhibitory actions of thalidomide. Hence, neither opening of K_{ATP} channel nor increasing cAMP levels explains the actions of thalidomide.

In further interaction studies, the p38 MAP kinase inhibitor SB203580, in concentrations reported to be effective (Seto *et al.*, 2006), failed to affect the inhibitory actions of thalidomide in epididymal portions. SB203580 produced a small but non-significant reduction in stimulation-evoked contractiosn in prostatic but not epididymal portions. This may suggest a role for p38 MAP kinase in contractions of prostatic portions. However, because thalidomide has no effect on prostatic portions and still produced inhibition of contractions in epididymal portions in the presence of SB203580, inhibition of p38 MAP kinase cannot be its mechanism of action. In rat aorta SB203580 is reported to reduce sensitivity to phenylephrine (Summers *et al.*, 2009).

Thalidomide also inhibits contractions in mouse vas deferens, producing significant inhibition at 10 μM. No further studies were carried out in mice, so that these results merely demonstrate that inhibitory effects of thalidomide are not restricted to rat vas deferens, although nothing can be said of the mechanism of action in mouse.

Thalidomide was found to mimic the T-type calcium channel blockers in that it inhibited contractions in epididymal portions of rat vas deferens but not in prostatic portions. Indeed, thalidomide had higher potency, producing inhibition at 10 μM, and was possibly more selective for epididymal over prostatic, and therefore possibly for T- over L-type channels, than mibefradil or NNC 55-0396.

We must consider how this work in rat vas deferens differs from and supplements that of Shishido *et al.* (2009) in guinea-pig vas deferens. The present study confirms that T-type calcium channel blockers have similar actions in rat and guinea-pig vas deferens. In the present study we have been able to clearly separate, anatomically, effects on L-type calcium channels from putative effects on T-type channels. This is of particular importance as our results would suggest

very limited selectivity of the two T-type channel antagonists, mibefradil and NNC-55-0396, in our functional studies. The contrast between the effects of the L-type channel blocker nifedipine and the T-type channel blockers is very stark. Nifedipine abolishes the L-type channel-mediated contraction in the prostatic portion without affecting the major adrenergic component of the contraction in the epididymal portion, whereas the T-type antagonists block contractions in the epididymal portion more than they block contractions in the prostatic portion. In the guinea-pig vas deferens with trains of pulses at 40 Hz, suramin or α,β-methylene-ATP was necessary to block the purinergic component of the contraction, which still left a biphasic response, of which the second component was sensitive to nifedipine (Shishido *et al.*, 2009). It should be noted that the two phases of response reported for trains of pulses in the guinea-pig vas deferens differ from those reported to single pulse stimulation in rat vas deferens. In guinea-pig vas deferens, the initial peak to trains of pulses occurs at 1.5 s and contains both purinergic and adrenergic components. The later peak to trains of pulses occurs at 5 s and may be due to the spillover of transmitter to sites more distant from the nerve endings. Nifedipine inhibited the late phase (at 5 s) but potentiated the early phase, and T-channel blockers inhibited both phases but were more potent at inhibiting the late (nifedipine-sensitive) phase, suggesting that they show some T-type selectivity (Shishido *et al.*, 2009).

One caveat in this study is that although two T-type calcium channel blockers, mibefradil and NNC 55-0396, block responses to nerve stimulation in epididymal portions of rat vas deferens, it is possible that they act at a site other than the T-channel.

Nifedipine induces penile erection in dogs by its vascular actions (Sarikaya *et al.*, 1997), and similar effects are reported in man (Kroner *et al.*, 1993). However, contractions of vas deferens to nerve stimulation are reduced to 60% of normal in mice lacking the P2X1 receptor for ATP, and fertility is reduced by 90% (Mulryan *et al.*, 2000). Because nifedipine blocks the purinergic component to the contraction of rat vas deferens and so acts functionally equivalent to a P2X1 receptor knockout, it is perhaps surprising that there are few reports of infertility with nifedipine (Enders, 1997). Little is known of the effects of T-type channel blockers on fertility.

In conclusion, the T-type calcium channel blockers mibefradil and NNC 55-0396 block particularly the adrenergic, nifedipine-resistant response to nerve stimulation in rat vas deferens, and this may suggest that this component of the contraction involves T-type calcium channels. In addition, thalidomide has actions that resemble those of the T-type calcium channel blockers, in that it blocks nifedipine-insensitive contractions in epididymal portions of rat vas deferens.

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