Differential effects of calcium channel blockers on the responses of the rat vas deferens to intramural nerve stimulation and exogenous drugs

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Summary. The calcium channel blockers, nifedipine and verapamil, have separate effects on the phases of nerve-induced twitches which are not reflected by their actions on the responses to exogenous NA, ATP and the stable ATP analogue, α,β-mATP. This implies that different calcium channels are used according to the manner of stimulation.

Key words. Post tetanic potentiation; vas deferens; smooth muscle; cotransmission; noradrenaline; nifedipine; neuromodulation; α-adrenoceptors.

Recent electrophysiological studies have established the existence of a variety of Ca^{2+} entry channels which differ in their activation, transient or long-lasting, and drug sensitivity[1,2]. However their relative contributions, and the diverse effects of different Ca^{2+} channel blockers, is poorly understood[3]. Here we show that the type of channel used depends on the mode of stimulation, neural stimulation of the vas deferens differing from bath application of the neurotransmitters.

The vas deferens appears to be activated by the sympathetic cotransmission of noradrenaline (NA) and adenosine 5′ triphosphate (ATP). The twitch response to a single shock is biphasic with the slow phase being abolished by α_1-adrenoceptor blockers[4] whilst the Ca^{2+}-channel blocker nifedipine selectively abolishes the fast phase[5]. Surprisingly nifedipine also abolishes the contractile response to exogenous NA[6]. Recent work suggests that the non-adrenergic phase of the twitch results from the action of ATP coreleased with NA on post-synaptic P_2-purinoceptors[7,8], and that this effect is mimicked by exogenous ATP[9]. The present study examines the effects of nifedipine and verapamil on the responses to intramural nerve stimulation, exogenous NA, ATP and α,β-mATP.

Materials and methods. The epididymal portions of the rat vasa deferentia, isolated from Lister rats (300–400 g) killed by a blow on the head, were desheathed using a light microscope (magnification: ×20). The tissues were mounted in a horizontal organ bath (resting tension 1 g) and superfused with Krebs solution (35–36 °C) at 5 ml/min. The preparation was stimulated using a Palmer square wave stimulator triggered by a microcomputer (Commodore Series 2001). A pair of parallel stainless steel electrodes (cross sectional area circa 1 mm^2) was mounted vertically on the moveable arm of an X-Y positioner. This arrangement allowed for localised field stimulation (70–90 V: 0.1 ms) of discrete regions of the tissue; in these experiments 10–15 mm from the epididymal end. Isometric tension was recorded via a Washington type D tension transducer on a Venture Servoscribe

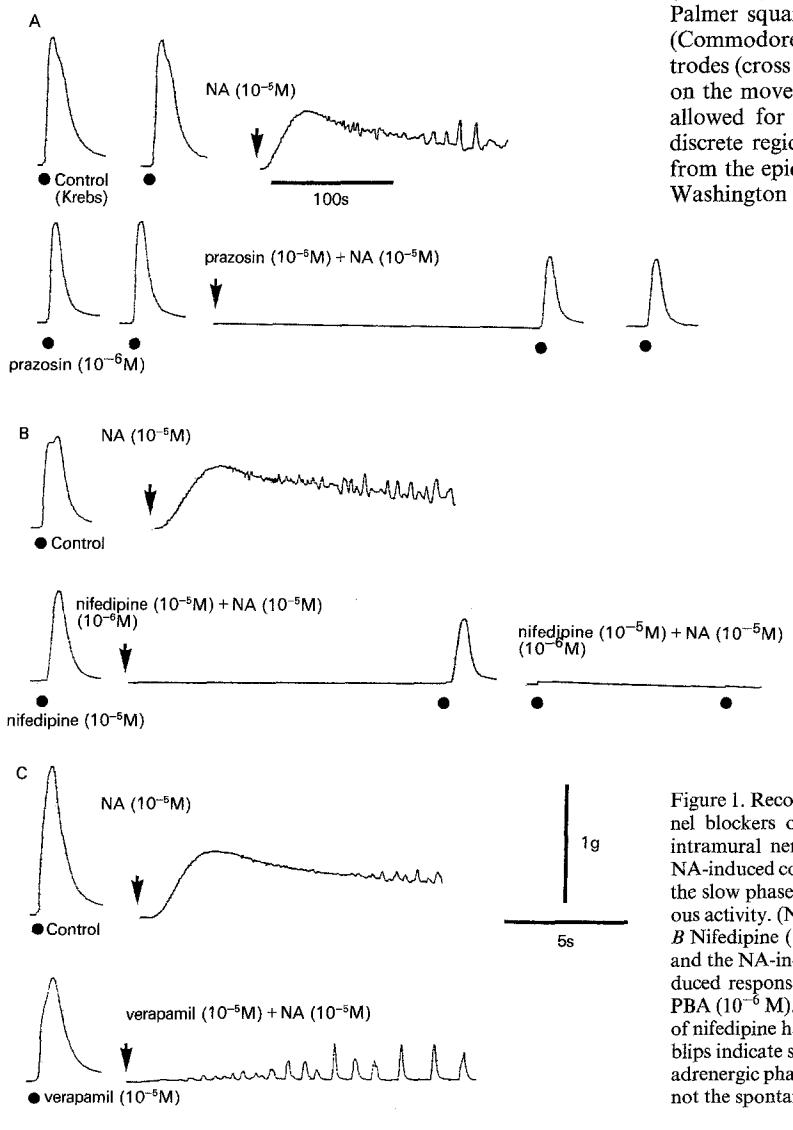

Figure 1. Records showing the effects of α_1-adrenoceptor and Ca^{2+}-channel blockers on the biphasic twitch responses of rat vas deferens to intramural nerve stimulation (indicated by dots) (5 s time scale) and NA-induced contraction (100 s time scale). *A* Prazosin (10^{-6} M) abolished the slow phase of the twitch, the NA-induced contraction and spontaneous activity. (Note that prazosin uncovers presynaptic inhibition by NA.) *B* Nifedipine (10^{-5} M) abolished the non-adrenergic phase of the twitch and the NA-induced contraction and spontaneous activity. The nerve-induced response remaining in the presence of nifedipine is abolished by PBA (10^{-6} M). The adrenergic phase of the twitch as seen in the presence of nifedipine has a longer latency than the non-adrenergic phase (upward blips indicate stimulus artefacts). *C* Verapamil (10^{-5} M) reduced only the adrenergic phase of the twitch, abolished the NA-induced contraction but not the spontaneous activity.

potentiometric recorder. The preparation was allowed to equilibrate for 30 min before the experiment. Stock solutions of NA (Sigma), α,β-methylene ATP (Sigma), ATP (Sigma), 8-phenyltheophylline (Sigma) and verapamil (Abbot Laboratory) were made up in Krebs. Phenoxybenzamine (Smith, Kline & French), prazosin (Pfizer) and nifedipine (gift from Dr. J.C. McGrath) were dissolved in absolute alcohol (0.1% v/V). Aliquots of all drugs were diluted to the required dose with Krebs before being used in the experiment. A 20 min contact time was allowed. With the stimulus parameters used all responses were abolished by tetrodotoxin (0.2 μg/ml), guanethidine or bretylium (10^{-5} M), indicating that the responses resulted from the stimulation of intramural nerves with the characteristics of adrenergic neurons.

Results. A) twitch phases and NA-induced contraction. Single shocks elicited biphasic twitches with time to peaks of 250–300 ms and 600–650 ms respectively (fig. 1A, B). In many preparations the phases were merged such that the twitch responses were monophasic with time to peak of 600–650 ms (fig. 1C). Exogenous NA ($5 \times 10^{-6} - 10^{-5}$ M) induced the development of a contractile response accompanied by spontaneous activity (fig. 1A). In the presence of NA both phases of the twitch were potentiated (fig. 2A). The α_1-adrenoceptor antagonist prazosin abolished the slow phase of the twitch (fig. 1A). It also abolished the NA-induced contraction, spontaneous activity and twitch potentiation (fig. 2A). Phenoxybenzamine (PBA) (10^{-6} M) and thymoxamine (10^{-6} M) had similar effects, although the NA-induced spontaneous activity was resistant to thymoxamine even when the dose was increased to 5×10^{-6} M. These results indicate that the slow phase of the twitch and the responses to exogenous NA result from the activation of post-synaptic α_1-adrenoceptors.

The effects of nifedipine (10^{-5} M) and verapamil (10^{-5} M) on the phases of the twitch and NA-induced responses are shown in figure 1B and C respectively. Nifedipine abolished the NA-induced contraction and spontaneous activity and the non-adrenergic rapid phase of the twitch but not its adrenergic phase. Subsequent addition of PBA in the presence of nifedipine resulted in the abolition of this remaining phase. In contrast the non-adrenergic phase of the twitch was not affected by verapamil ($5 \times 10^{-6} - 10^{-5}$ M) but the adrenergic slow phase was reduced (by 30.5 ± 1.46%, n = 8, at 10^{-5} M). Figure 1C also shows that the NA-induced contraction but not the spontaneous muscle activity was abolished by verapamil.

B) response to α,β-mATP and ATP. Exogenous α,β-mATP (3×10^{-6} M – 10^{-5} M) produced a dose related increase in muscle tone followed by complete relaxation attributed to the desensitization of the post-synaptic P_2-purinoceptors[10] (fig. 2B). After this the continued presence of α,β-mATP (10^{-5} M) resulted in the reduction of both phases of the twitch (fig. 2A, B). Perfusion with α,β-mATP and PBA or prazosin, for up to 30 min, resulted only in the abolition of the slow phase (fig. 2B, C). Doses of nifedipine ($5 \times 10^{-6} - 10^{-5}$ M) which abolished the non-adrenergic phase of the twitch produced a 47.0 ± 7.3% (n = 3) reduction in the peak size of the contraction induced by 3×10^{-6} M α,β-mATP. In two preparations the maximum size of the contraction to α,β-mATP was unaffected by nifedipine (fig. 2C) but the decline was faster. Surprisingly doses of verapamil (10^{-5} M) which had no effect on the non-adrenergic phase of the

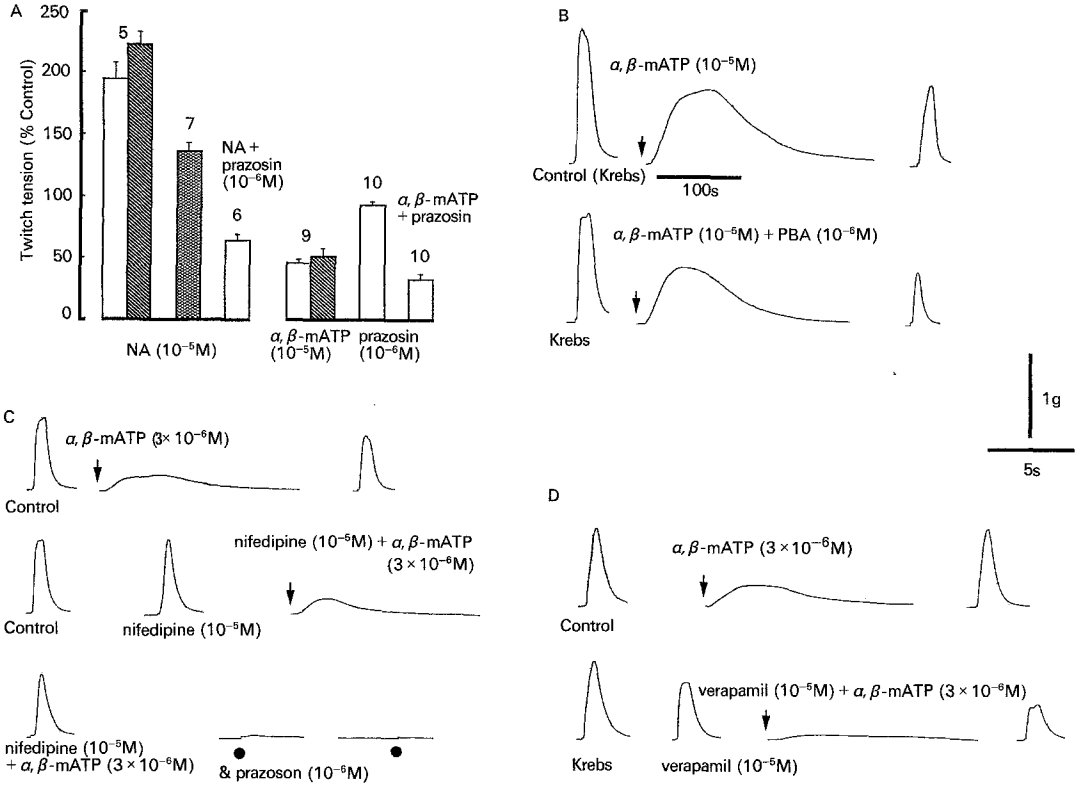

Figure 2. *A* Histogram of twitch responses relative to Krebs showing the NA-induced potentiation and α,β-mATP-induced inhibition. Non-adrenergic and adrenergic phases of the twitch are shown in open and hatched columns respectively; cross-hatched column shows the mixed monophasic response. Prazosin (10^{-6} M) has no effect on the non-adrenergic phase (fourth open column) but abolished the adrenergic phase of the twitch, the NA-induced potentiation and deepened the α,β-mATP-induced twitch depression. Bars indicate ±SEM. Numbers on columns represent number of preparations. *B–D* Each panel shows sequential records from a single preparation. Transient contractile response induced by α,β-mATP (10^{-5} M) (100 s time scale) and its effects on the phases of the twitch (5 s time scale) recorded in the presence of *B* Krebs, *C* nifedipine (10^{-5} M) and prazosin (10^{-6} M) and *D* verapamil (10^{-5} M). Note in *B*: PBA had no effect on the α,β-mATP-induced contraction but abolished the adrenergic slow phase of the twitch.

twitch produced a 66.8 ± 5.1% (n = 4) depression of the contraction induced by α,β-mATP (fig. 2B).

The tissue was relatively insensitive to exogenous ATP, even high doses eliciting small responses. Exogenous ATP ($> 10^{-4}$ M) gave dose-related contractions which rose to a peak and subsequently declined during continuous perfusion. Subsequent single shocks in the presence of ATP elicited biphasic twitches with both phases significantly ($p < 0.01$) depressed relative to the controls. 5×10^{-4} M ATP depressed the nonadrenergic and adrenergic phases of the twitch by 77.1 ± 4.9% and 80.2 ± 3.8% (n = 6) respectively. This depression was not prevented by 8-phenyltheophylline (10^{-5} M), a presynaptic P_1-purinoceptor antagonist[11]. This suggests that the P_2-purinoceptors are rapidly desensitized on exposure to ATP[12].

Nifedipine or verapamil did not abolish the ATP-induced contraction. In the presence of nifedipine (10^{-5} M) or verapamil (10^{-5} M) the peak size of the contraction to ATP (5×10^{-4} M) was reduced by 51.7 ± 5.2% (n = 5) and 32.7 ± 1.8% (n = 4) respectively. This result contrasts with the effect of these calcium channel blockers on the non-adrenergic phase of single shock responses.

Discussion. In different tissues including the rat vas deferens, nifedipine and verapamil abolish responses which result from the activation of voltage-sensitive Ca^{2+}-channels[13–15]. The results presented in this paper demonstate clearly that transmitters released neurally, compared to exogenous application, have different sensitivities to Ca^{2+}-channel blockers. Although the same post-junctional α_1-adrenoceptors mediate the responses to neurally released NA and to bath applied NA, only the latter is abolished by nifedipine. This suggests that whilst the NA-induced contraction depends on Ca^{2+} influx through nifedipine sensitive Ca^{2+}-channels, the adrenergic slow phase of the twitch probably develops in response to the release of sequestered Ca^{2+}[14]. Replacement of Ca^{2+} with Sr^{2+}, which supports transmitter release, results in the marked depression and subsequent inhibition of only the adrenergic slow phase[16], presumably because the sequestered pool is not being replenished. Furthermore the longer latency of the adrenergic slow phase[17] indicates the involvement of secondary internal processes. This process, although requiring Ca^{2+}, may use a different tension generating mechanism such as myosin phosphorylation[18].

The non-adrenergic rapid phase of the twitch, recently attributed to the action of neurally released ATP on post-synaptic P_2-purinoceptors[8], is abolished by nifedipine but not by verapamil. In contrast the activation of these purinoceptors with ATP or its stable analogue α,β-mATP elicits a contractile response that is not abolished or at best is only partially sensitive to either Ca^{2+}-channel blocker. Again, the responses to neurally released and to exogenously applied transmitters depend on Ca^{2+} influx through different voltage-sensitive Ca^{2+}-channels.

A variety of Ca^{2+}-channels have been identified in different tissues including smooth muscles[2]. The Ca^{2+}-channels involved in spike generation activate and inactivate rapidly, others activate relatively slowly in response to sustained depolarization. Nifedipine selectively abolishes the generation of action potentials and the non-adrenergic phase of the twitch in the guinea pig and rat vasa deferentia[5], probably indicating the involvement of the rapidly activating Ca^{2+}-channels. Activation of post-synaptic P_2-purinoceptors or α_1-adrenoceptors by exogenous ATP or NA respectively produces a depolarization of the smooth muscle cells of the guinea pig vas deferens[19,20]. If this is also true of the rat then the contractions to exogenous ATP, α,β-mATP or NA, which are sensitive to verapamil as well as nifedipine, must result from Ca^{2+} influx through the slowly activating Ca^{2+}-channels. This is consistent with the finding that the influx of labeled Ca^{2+} and the contractions of the rat vas deferens induced by elevated KCl is abolished by both verapamil and nifedipine[21]. Likewise the susceptability of the adrenergic secondary component of the tetanic response contrasts with the relative insensitivity of the adrenergic phase of the twitch to nifedipine[6] and verapamil[22]. Thus where there is a sustained depolorisation the responses appear to depend on slowly activating Ca^{2+} channels which are sensitive to both verapamil and nifedipine. It remains a puzzle why doses of nifedipine which abolished the nonadrenergic phase of the twitch, supposedly purinergic, failed to block the response to exogenous ATP.

In conclusion these results demonstrate that the sources of Ca^{2+} utilized by the smooth muscle, and thus the effectiveness of Ca^{2+}-channel blockers, may depend not only on the transmitter involved but also on the stimulus duration and origin (i.e. neurally released or exogenously applied) of the agonist.

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Diltiazem prevents the damage to cultured aortic smooth muscle cells induced by hyperlipidemic serum

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Summary. Diltiazem, a calcium antagonist, significantly reduced the increased ^{45}Ca uptake and the number of dead cells in cultured aortic smooth muscle cells induced by hyperlipidemic serum.
Key words. Diltiazem; hyperlipidemia; cultured aortic smooth muscle.