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Selective blockade by nifedipine of 'purinergic' rather than adrenergic nerve-mediated vasopressor responses in the pithed rat

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1 Nifedipine can attenuate pressor responses to sympathetic nerve stimulation both in the presence and in the absence of α-adrenoceptor blocking agents.

2 In the presence of α,β-methylene ATP, nifedipine produces only a small attenuation of the vasopressor response.

3 Nifedipine attenuates the vasopressor response produced by intravenous bolus administration of α,β-methylene ATP.

4 The results suggest that the purinergic component of the vasopressor response to stimulation of the sympathetic outflow in the rat is subject to blockade by nifedipine, whereas the α-adrenoceptor-mediated response to co-transmitter noradrenaline is relatively resistant.

Introduction

Sequential administration of α-adrenoceptor antagonists has uncovered pressor nerve-mediated responses in the vasculature *in vivo* and *in vitro*, which are not mediated by α-adrenoceptors, and in several cases these have been shown to be susceptible to blockade by the P_{2x}-desensitizing agent α,β-methylene ATP (mATP) (Burnstock & Kennedy, 1985; Flavahan *et al.*, 1985; Grant *et al.*, 1985; Ishikawa, 1985; von Kugelen & Starke, 1985; Kennedy *et al.*, 1986; Muramatsu, 1986; Vidal *et al.*, 1986; Burnstock & Warland, 1987; Ramme *et al.*, 1987; Bulloch & McGrath, 1988), suggesting that they are purinergic.

Both mATP (Sneddon & Burnstock, 1984) and the slow Ca^{2+} channel blocker nifedipine (Blakeley *et al.*, 1981) block non-adrenergic excitatory nerve-mediated responses of the vas deferens. We now demonstrate that the purinergic/adrenergic co-transmission to vascular smooth muscle *in vivo* also shows differential susceptibility of the purinergic component to nifedipine.

Methods

Male Wistar rats (250 g) were pithed under halothane anaesthesia (Gillespie *et al.*, 1970), artificially ventilated with 40% oxygen and 60% nitrogen and given gallamine (10 mg kg^{-1}, i.v.) to stop skeletal muscle twitching and propranol (1 mg kg^{-1}, i.v.) to eliminate vasodilatation due to catecholamine release from the adrenal medulla ('endogenous adrenaline reversal') (Flavahan *et al.*, 1985). Drugs were administered via the right external jugular vein, except for nifedipine which was administered retrogradely via the carotid artery since intravenous administration stops the heart. Right carotid arterial blood pressure and heart rate, derived electronically from this signal, were monitored continuously: diastolic pressor responses to sympathetic nerve stimulation via the pithing rod were measured (1 cm electrode, T8, supramaximal voltage, 0.05 ms pulses, 1 s train, 5–20 Hz, Grass S88 stimulator) (Gillespie & McGrath, 1974).

The desensitization procedure for P_2-purinoceptors *in vivo* has been published (Bulloch & McGrath, 1988) and is summarized below:

An initial single bolus injection of 0.5 mg kg^{-1} mATP caused heart failure in the rats. Therefore an initial low dose of mATP of 0.05 mg kg^{-1} was introduced to desensitize the heart to this effect of mATP. A final cumulative dose of mATP of approximately 2.5 mg kg^{-1} was gradually introduced to the rat. The first 2 boluses of mATP were given at a dose level of 0.05 mg kg^{-1}. This was followed by 5 separate boluses of mATP at a dose level of 0.5 mg kg^{-1} approximately every 60 s. This desensitization procedure spanned a 7 min period.

Drugs used were gallamine triethiodide (Flaxedil) (May & Baker), halothane (Fluothane) (ICI), α,β-methylene-adenosine 5'-triphosphate (mATP,

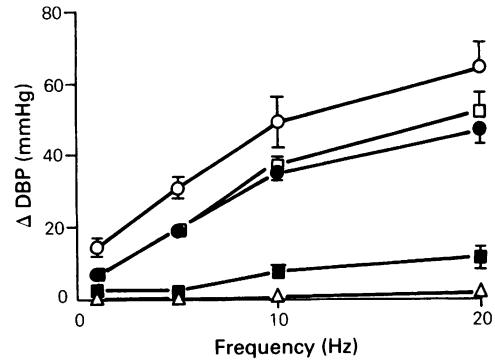

Figure 1 The effect of nifedipine on α-blocked vasopressor responses to stimulation of the sympathetic outflow T_6–T_8, for 1 s at 1–20 Hz, measured as changes in diastolic blood pressure (Δ DBP). Responses were measured after propranolol 1 mg kg^{-1} (○), then prazosin 1 mg kg^{-1} (●), rauwolscine 1 mg kg^{-1} (□), nifedipine 0.3 mg kg^{-1} i.a. (■) and finally α,β-methylene ATP* (△). (*according to desensitization schedule). Vertical lines indicate s.e.mean (n = 6).

Sigma), nifedipine (Bayer), (−)-noradrenaline bitartrate (Sigma), prazosin HCl (Pfizer), (±)-propranolol HCl (Sigma), rauwolscine HCl (Roth). The doses of all drugs are expressed as g of the salt per kg body

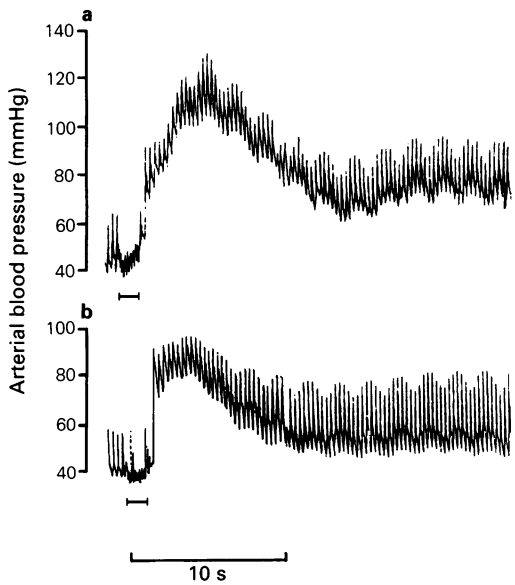

Figure 2 Arterial blood pressure recording from a pithed rat showing pressor responses obtained to stimulation of the sympathetic spinal outflow (|—|) T_6–T_8, 1 cm electrode, 0.05 ms pulse width, 1 s, 20 Hz. (a) In the presence of propranolol 1 mg kg^{-1} and gallamine 10 mg kg^{-1}; and (b) after nifedipine (i.a.) 0.3 mg kg^{-1}.

weight. All drugs were dissolved in 0.9% saline except mATP, prazosin and rauwolscine, which were dissolved in distilled water, and nifedipine, which was dissolved in polyethylene glycol and ethanol (1:4), before being diluted down in distilled water. Nifedipine solutions were protected from light and noradrenaline solutions were protected from oxidation by the inclusion of 23 μM ethylene diamine tetraacetic acid.

The means and s.e.mean for absolute values of responses obtained from groups of rats were calculated and where appropriate the means were compared by use of the paired t test. P values of 0.05 or less were considered to be significant. n is the number of preparations.

Results

Nerve mediated pressor responses

Stimulation of the spinal sympathetic outflow (T_1–L_4) via the pithing rod electrode (1 cm tip exposure) evoked pressor responses. The size and time course of these responses depended on the position of the stimulating electrode. The largest and most reproducible vasopressor responses with minimal cardioaccelerator effects were obtained when the pithing rod electrode tip was positioned between T_6 and T_8 but stimulation of any area within the region T_1–L_4 produced pressor responses. The vasopressor responses were frequency-dependent (Figure 1). Flavahan *et al.* (1985) showed that when the stimulating electrode is placed between regions T_3 and L_4, the resulting pressor response is biphasic and comprises an early direct pressor component and a delayed pressor component due to stimulation of the adrenals. Therefore, for the present study, which concerns the direct component, T_8 was chosen and responses were measured at the early peak following a short 1 s train of pulses.

Responses to sympathetic nerve stimulation were blocked only partially by the administration of the α-adrenoceptor antagonists prazosin and rauwolscine together (each 1 mg kg^{-1}) (Figure 1) but were completely blocked by a combination of mATP, prazosin and rauwolscine (Bulloch & McGrath, 1988).

At stimulation frequencies of 1 to 20 Hz, after the administration of prazosin and rauwolscine, the remaining direct component of the pressor response was further attenuated by the intra-arterial administration of nifedipine (0.3 mg kg^{-1}), leaving a small pressor response which was approximately 15% of the control level. The blockade by the α-adrenoceptor antagonists was frequency-dependent, causing the greatest proportionate reduction of responses at lower frequencies but the blockade of

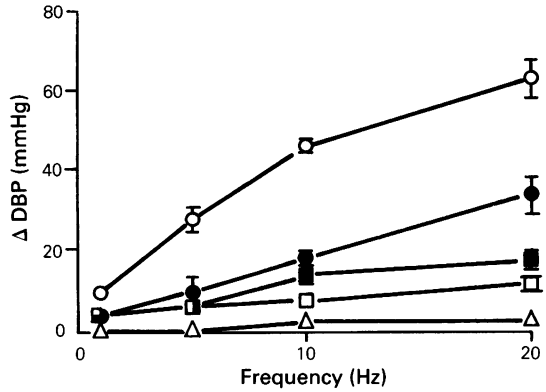

Figure 3 The effect of nifedipine on unblocked vasopressor responses to stimulation of the sympathetic outflow T_6–T_8, for 1 s at 1–20 Hz, measured as changes in diastolic blood pressure (Δ DBP). Responses were measured after propranolol 1 mg kg^{-1} (○), then nifedipine 0.3 mg kg^{-1} i.a. (●), prazosin 1 mg kg^{-1} (□), rauwolscine 1 mg kg^{-1} (■), and finally α,β-methylene ATP (△). Vertical lines indicate s.e.mean (n = 6).

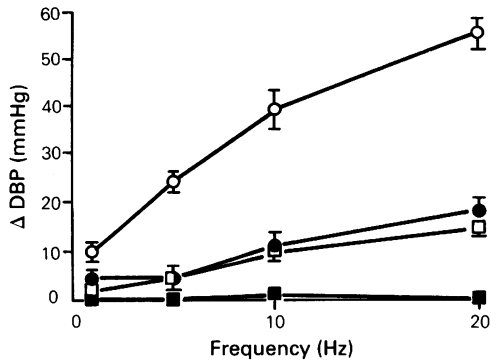

Figure 4 The effect of nifedipine on P_2-purinoceptor desensitized vasopressor responses to stimulation of the sympathetic outflow T_6–T_8, for 1 s at 1–20 Hz, measured as changes in diastolic blood pressure (Δ DBP). Responses were measured after propranolol 1 mg kg^{-1} (○), then α,β-methylene ATP (●), nifedipine 0.3 mg kg^{-1} i.a. (□), and finally prazosin 1 mg kg^{-1} and rauwolscine 1 mg kg^{-1} together (■). Vertical lines indicate s.e.mean (n = 6).

the residual response by nifedipine occurred evenly throughout the frequency range (Figure 1). The subsequent administration of mATP, sufficient for desensitizing P_{2x}-purinoceptors, eliminated the remaining pressor response at frequencies of 1–10 Hz and left a vasopressor response of less than 5 mmHg at 20 Hz (approximately 6% of control response).

The blocking effect of nifedipine was seen also without the administration of the α-adrenoceptor antagonists (Figures 2 and 3). Subsequent administration of prazosin attenuated the response by about 60%, indicating that a large part of the response remaining after nifedipine was mediated by α_1-adrenoceptors. A subsequent dose of rauwolscine produced a small increase in the size of the responses (significant at frequencies of 10 and 20 Hz) suggesting some control of the residual purinergic response by prejunctional α_2-adrenoceptors.

After the administration of a P_{2x}-purinoceptor desensitizing dose of mATP, nifedipine had very little effect on the vasopressor response. After the addition of mATP and nifedipine, the subsequent administration of prazosin (1 mg kg^{-1}) and rauwolscine (1 mg kg^{-1}) together, almost completely blocked the pressor responses at all frequencies (Figure 4).

The effect of nifedipine on pressor responses to α,β-methylene ATP

The intravenous administration of mATP (0.01 mg kg^{-1}–0.5 mg kg^{-1}) produced large but short-lived pressor responses. The first few additions produced pressor responses that were biphasic and hence similar in appearance to responses mediated by either NA or nerve stimulation. The first, but not the subsequent, doses produced increases in heart rate, which presumably contribute to the complex first pressor response. mATP administration always resulted in pressor responses even when vascular tone was raised by infusing NA (0.1 to 1 μg min^{-1}, i.v.). Since mATP responses were subject to tachyphylaxis, it was not possible to construct a dose-response curve in a single preparation. Instead a comparison of pressor responses to first additions of mATP was made between animals (Figure 5). Prazosin did not appear to alter these responses, whereas pretreatment with nifedipine (0.3 mg kg^{-1}) resulted in pressor responses to mATP being smaller than in controls, although neither antagonist altered the biphasic nature of the mATP response or the effect on heart rate.

Discussion

Our earlier findings showed that the widespread sympathetic vasopressor response to stimulation of the sympathetic outflow in the rat is due to adrenergic and purinergic elements acting as co-transmitters, since the whole response is blocked by chemical sympathectomy or guanethidine, part is blocked by reserpine or α-adrenoceptor antagonists and the other part is blocked by mATP, the purinoceptor (P_{2x}) desensitizing agent. The responses are

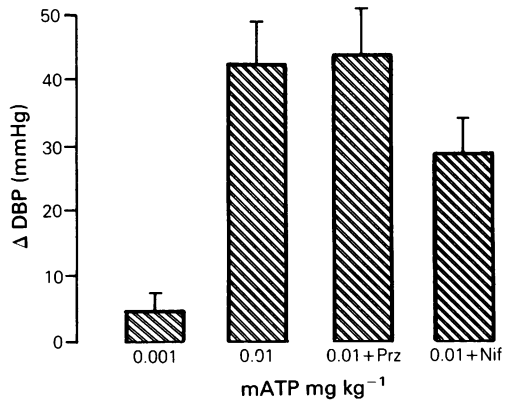

Figure 5 The effects of prazosin and nifedipine on the first pressor response to α,β-methylene ATP (mATP). First two columns are controls to intravenous administration of 0.001 mg kg^{-1} mATP and 0.01 mg kg^{-1} mATP; third and fourth columns show responses to 0.01 mg kg^{-1} mATP in the presence of prazosin 1 mg kg^{-1} (Prz) or nifedipine 0.3 mg kg^{-1}. (Nif). All data were obtained from different animals to overcome problems of tachyphylaxis. Bars indicate s.e.mean. ($n = 4$ for each column). * Significantly different from 0.01 control, $P < 0.05$.

essentially additive and can be attenuated by sequential administration of the selective blockers, irrespective of the order of administration (Flavahan *et al.*, 1985; Grant *et al.*, 1985; Bulloch & McGrath, 1986; 1988).

This mirrors the pharmacological properties of the co-transmission in the vas deferens of various species both *in vitro* (Sneddon & Burnstock, 1984) and *in situ* (Bulloch & McGrath, 1988). Electrophysiological analysis of transmission in the guinea-pig vas deferens showed that the transduction process for the purinergic component involved excitatory junction potentials in the smooth muscle cells, which summated to fire action potentials when a threshold membrane depolarization was achieved (Burnstock & Holman, 1964; Burnstock & Sneddon, 1985). This action potential propagates by means of the slow Ca^{2+} channel (Bennett, 1967; Abe, 1968; Brading *et al.*, 1969). Consequently the purinergic-mediated non-adrenergic contraction in response to sympathetic nerve activation depends on the functioning of this channel and if it is blocked by nifedipine then the action potential is lost and the purinergic contraction is blocked (Blakeley *et al.*, 1981).

In contrast, with single stimuli or short trains of pulses, the adrenergic response is unaffected by the same dose of nifedipine (Blakeley *et al.*, 1981). The present study shows that exactly the same occurs with short trains of vasopressor stimuli. The mATP-sensitive, purinergic component is blocked but the adrenergic response is unaffected after nifedipine. This suggests that the model of α-adrenoceptor/P_{2x}-purinoceptor-mediated co-transmission found in the vas deferens applies also to co-transmission in blood vessels (Figure 6).

This has considerable significance for the interpretation of the cardiovascular effects of drugs affecting smooth muscle Ca^{2+} channels. With the short bursts of activity which characterize sympathetic nerves *in vivo* it seems likely that drugs which block the slow Ca^{2+} channel will selectively block the purinergic element leaving the adrenergic part. Thus in any particular disease state or with multiple drug therapy, residual sympathetic control of blood pressure will depend on the functional (or pharmacological) state of the α-adrenoceptor system. Also, the model implies that the purinergic response is propagated whereas the adrenergic response is not. In the vas deferens, this helps to explain why the purinergic component is relatively resistant to extensive chemical sympathectomy (Brown *et al.*, 1983). Chemical sympathectomy does in fact eliminate the vasopressor nerve-mediated response (Flavahan *et al.*, 1985), but clearly the greater density of innervation in the vas deferens partly accounts for its particular resistance. Thus, it seems possible that propagation of the purinergic component will occur in blood vessels and this will have many implications for the different physiological roles of the two co-transmitters.

α-Adrenoceptor-mediated pressor responses to circulating agonists are partly affected by Ca^{2+} antagonists such as nifedipine (Timmermans *et al.*, 1983), but this varies with the agonist and method of administration and is partly attributable to the duration of the response (McGrath & O'Brien, 1987). With sympathetic nerve-mediated responses in different preparations, any variability in blockade is likely to be at least partly attributable both to variations in the contributions from purinergic and adrenergic transmission and to the duration of the stimulus and/or the response. The present results apply only to short trains of pulses. In the vas deferens the α-adrenoceptor-mediated responses to long trains of pulses are susceptible to nifedipine and thus appear to involve a different excitation-contraction coupling process (Brown *et al.*, 1983). By analogy, the same might apply to blood vessels. In the dog isolated mesenteric artery the early part of the response to transmural nerve stimulation contains a relatively greater purinergic component than does the later part of the response (Dalziel *et al.*, 1987), so it will be interesting to see whether nifedipine can further dissect the adrenergic component as it can in vas deferens (Blakeley *et al.*, 1981). It is already known that nifedipine has differential effects on the early and late components of α-adrenoceptor-mediated

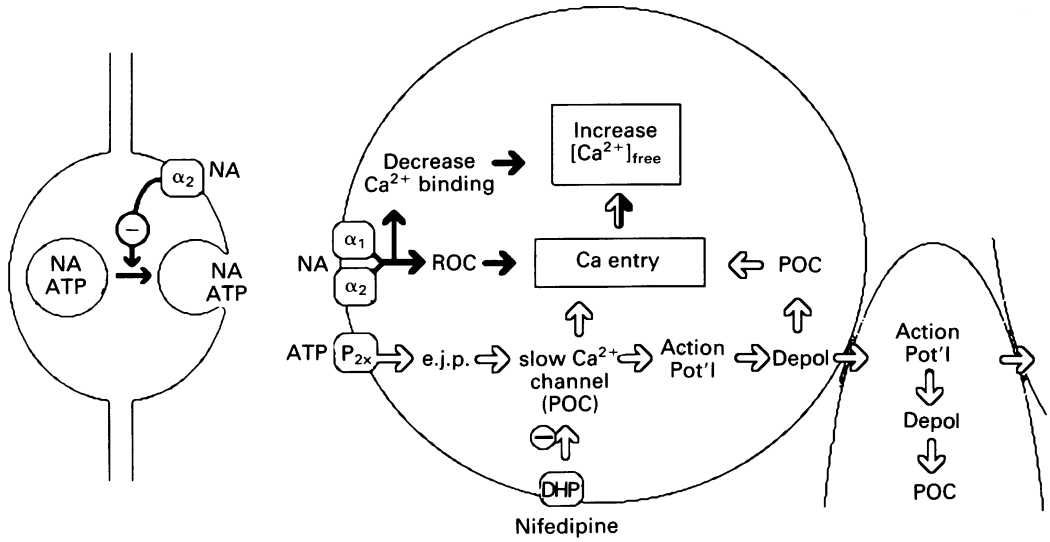

Figure 6 Hypothetical representation of the different coupling mechanisms for purinergic and adrenergic components of the contractile response to sympathetic nerve stimulation in smooth muscle of the vas deferens and vasculature (with single pulses or short trains). The postjunctional α-adrenoceptors, which are exclusively α_1 in vas deferens, but include also α_2 in blood vessels, utilize coupling pathways unaffected by nifedipine, which could include release of intracellular Ca^{2+} or dihydropyridine-resistant receptor operated Ca^{2+} channels (ROC): this is unresolved. The P_{2x}-purinoceptors open ion channels to produce depolarization which becomes apparent as the excitatory junction potential (e.j.p.) and this in turn initiates the slow Ca^{2+} channel-borne spike of the action potential. Nifedipine blocks the sequence at this point (Blakeley *et al.*, 1981) by an inhibitory action on channel opening exerted through dihydropyridine binding sites (DHP). In the absence of blockade, the action potential will be propagated throughout the cell and, through tight junctions, to other cells, initiating as it goes the opening of potential operated Ca^{2+} channels (POC) which lead to increased intracellular $[Ca^{2+}]_{free}$ and thus contraction. Nifedipine could also block the opening of these POCs, which are not distinguishable from the channels opened by the e.j.p.

pressor responses to exogenous noradrenaline (NA) or other agonists: the initial, early, rapid responses are resistant but the late components or responses to infusion are blocked (Grant *et al.*, 1985; McGrath & O'Brien, 1987).

The pressor response to mATP, like the α-blocker-resistant response to nerve stimulation, was itself resistant to prazosin but susceptible to nifedipine. This is consistent with the hypothesis that it acts as a desensitizing agonist at the same P_{2x}-receptors that mediate the purinergic component of the pressor nerve-induced response.

The administration of rauwolscine highlighted the potential difficulties encountered when using an antagonist which is not selective for postsynaptic α_2-adrenoceptors only. Rauwolscine's action on the presynaptic nerve terminal led to increases in the height of the responses by interfering with the feedback system. This therefore complicates interpretation of results since blockade of α_2-adrenoceptors should reduce autofeedback and increase release of both transmitters, enhancing purinergic and adrenergic contributions to transmission. Therefore in the presence of both prazosin and rauwolscine an exaggerated purinergic component should be observed. There was some evidence of this in individual experiments when rauwolscine was given after prazosin. The action of rauwolscine also showed the problems faced when analysing results merely by measuring maximum heights of the responses rather than analysing the shape of the response (i.e. its time course), since it prolonged the early direct component as well as increasing its height (quantitative results not shown).

All of the data from the pithed rat are now consistent with the hypothesis that NA and ATP can act as co-transmitters at vascular neuroeffector junctions and produce additive responses through only α-adrenoceptors and P_{2x}-purinoceptors, respectively, and that responses mediated via P_2-purinoceptors can be antagonized selectively by nifedipine.

J.M.B. held an SERC CASE studentship in collaboration with Pfizer Central Research.

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