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Blockade of vasopressor and vas deferens responses by α, β-methylene ATP in the pithed rat

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1 The pressor responses produced by the intravenous administration of α,β-methylene ATP were tachyphylactic.

2 α,β-Methylene ATP can attenuate pressor responses to sympathetic nerve stimulation both in the presence and in the absence of α-adrenoceptor blocking agents.

3 α,β-Methylene ATP has no effect on the pressor responses produced by bolus injections of noradrenaline.

4 In the presence of α-adrenoceptor blocking agents, α,β-methylene ATP further attenuates contractions of the vas deferens produced by nerve stimulation.

5 The results, together with previous data, suggest that the vasopressor response to stimulation of the sympathetic outflow in the rat is partly purinergic and partly α-adrenergic and that this occurs as co-transmission. The same applies to rat vas deferens, confirming *in vitro* data. The pithed rabbit had an α-blocker-resistant vasopressor nerve-mediated response but this was resistant to α,β-methylene ATP.

Introduction

Since it was first proposed that cotransmission involving adrenergic and purinergic elements may occur in some vascular smooth muscle (Sneddon & Burnstock, 1984), further evidence for this phenomenon has been reported both *in vitro* (Ishikawa, 1985; von Kugelen & Starke, 1985; Cheung & Fujioka, 1986; Kennedy *et al.*, 1986; Muramatsu, 1986; Vidal *et al.*, 1986; Burnstock & Warland, 1987; Ramme *et al.*, 1987) and *in vivo* (Flavahan *et al.*, 1985; Grant *et al.*, 1985).

In previous studies using the pithed rat, we demonstrated that the P_{2x}-purinoceptor desensitizing agent, α,β-methylene ATP (mATP) (Burnstock & Kennedy, 1985) could attenuate pressor responses to sympathetic nerve stimulation after blockade of α-adrenoceptors but not in the absence of α-blockade (Flavahan *et al.*, 1985; Grant *et al.*, 1985). This seemed to suggest a relatively minor role for purinergic co-transmission in rat vasculature. We now show that by adopting a novel desensitization schedule for mATP and taking into account the short life of mATP *in vivo*, pressor responses due to sympathetic nerve stimulation can be shown to be more sensitive to mATP than was previously suggested. In order to verify our interpretation, we also monitored tension in the vas deferens *in vivo* since it is well established *in vitro* that this organ has adrenergic/purinergic cotransmission (Sneddon *et al.*, 1983; Meldrum & Burnstock, 1983; Sneddon & Burnstock, 1984; Sneddon & Westfall, 1984; Stjärne & Astrand, 1984; 1985).

A preliminary communication of these results has been published (Bulloch & McGrath, 1986).

Methods

Male Wistar rats (250 g) were pithed under halothane anaesthesia (Gillespie *et al.*, 1970) and were artificially ventilated with 40% oxygen and 60% nitrogen and given gallamine (10 mg kg^{-1}, i.v.) to stop skeletal muscle twitching and propranolol (1 mg kg^{-1}, i.v.) to eliminate vasodilatation due to catecholamine release from the adrenal medulla or 'endogenous adrenaline reversal' (Flavahan *et al.*, 1985). Drugs were administered via the right external jugular vein. 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 (1 cm electrode, T_8, 1 s, 5–20 Hz) were measured.

Longitudinal tension responses of the vasa deferentia were recorded *in situ* by the method employed by Gillespie & McGrath (1974) to stimulation of the spinal sympathetic outflow from the pithing rod electrode (1 cm electrode, T_{13}, 1 s, 5–20 Hz).

The desensitization procedure for P_2-purinoceptors *in vivo* was as follows: An initial single bolus injection of mATP, 0.5 mg kg^{-1}, 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.

Male New Zealand white rabbits (3–4 kg) were pithed under halothane anaesthesia (McGrath & McKenzie, 1977) then artificially ventilated with 100% oxygen. The left carotid artery was cannulated to monitor arterial blood pressure. Drugs were administered via a cannula inserted into the right external jugular vein. Propranolol (1 mg kg^{-1}, i.v.) was given at 30 min intervals. The diastolic pressor effects of sympathetic nerve stimulation via the pithing rod (1 cm electrode, T_8, 1–200 pulses, 1–20 Hz) were determined under control conditions, following administration of α-adrenoceptor antagonists and finally after the same dose schedule of mATP as used in the rat.

Drugs used were gallamine triethiodide (Flaxedil) (May & Baker), halothane (Fluothane) (ICI), α,β-methylene-adenosine 5′-triphosphate (Sigma), noradrenaline bitartrate (Sigma), prazosin HCl (Pfizer), propranolol HCl (Sigma), rauwolscine (Roth). The doses of all drugs are expressed as g of the salt per kg body weight.

The means and standard errors of the mean for the groups of experiments shown in this paper were calculated and the means were compared using the paired *t* test. *P* values of 0.05 or less were considered to be significant. *n* is the number of preparations.

Results

Effects of antagonists on pressor responses to stimulation of spinal sympathetic outflow

The i.v. administration of mATP (0.01–0.5 mg kg^{-1}) produced a short-lived pressor response (< 10 s) which was subject to tachyphylaxis (Figure 1). The pressor response to the first dose of mATP given in each experiment irrespective of dose within the range tested differed from the subsequent pressor responses to mATP. The first response was biphasic having an immediate transient short-lived pressor effect and a second longer lasting pressor phase. It was also accompanied by an increase in heart rate. Subsequent doses produced a monophasic pressor response equivalent to the second phase and did not alter heart rate. After desensitization the pressor responses were not significantly larger than responses to the equivalent volume of 0.9% saline.

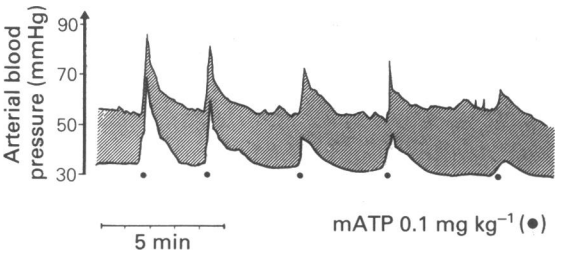

Figure 1 Recording of arterial blood pressure monitored from the carotid artery and showing the effect of intravenous administration of boluses of α,β-methylene ATP (mATP, 0.1 mg kg^{-1}). The first bolus of mATP produces a large pressor response but subsequent administrations of the same dose showed tachyphylaxis.

Following completion of the desensitization schedule for mATP, attenuation of nerve-mediated pressor responses occurred in a time-dependent manner: maximum blockade occurred 1 min after the addition of mATP but faded within 10 min to a maintained desensitization (Figure 2).

Responses to sympathetic nerve stimulation were only partially blocked by the administration of the

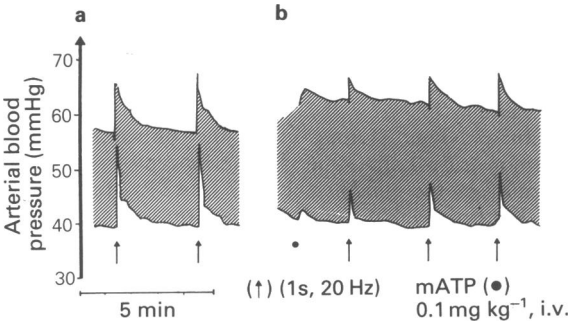

Figure 2 Recording of carotid arterial blood pressure showing the effect of stimulation (↑) of the spinal sympathetic outflow (T_6–T_8) for 1 s at 20 Hz, in (a) control conditions (in the presence of propranolol (1 mg kg^{-1}) and gallamine (10 mg kg^{-1}) only); and (b) after additions of α,β-methylene ATP (mATP) to produce desensitization of the P_2-purinoceptors. The final addition of mATP (0.1 mg kg^{-1}) in the desensitizing schedule is also indicated on the trace (●).

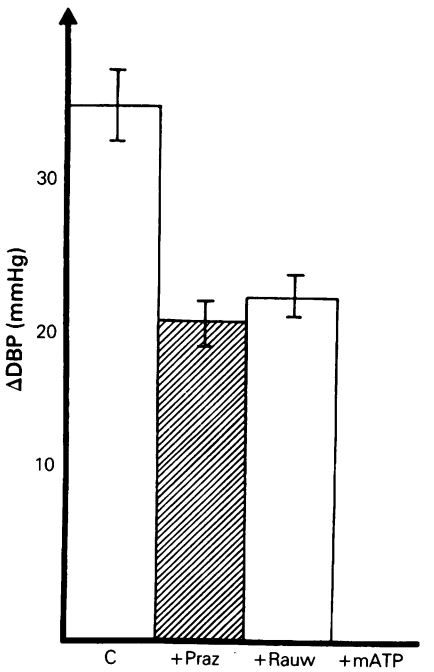

Figure 3 Peak vasopressor responses to sympathetic nerve stimulation (T_6–T_8) for 1 s at 20 Hz were measured as changes in diastolic blood pressure (ΔDBP) in control conditions (in the presence of propranolol (1 mg kg^{-1}) and gallamine (10 mg kg^{-1})) and after the sequential administration of antagonists; prazosin (Praz, 1 mg kg^{-1}), rauwolscine (Rauw, 1 mg kg^{-1}), and a desensitizing dose of α,β-methylene ATP (mATP). $n = 6$

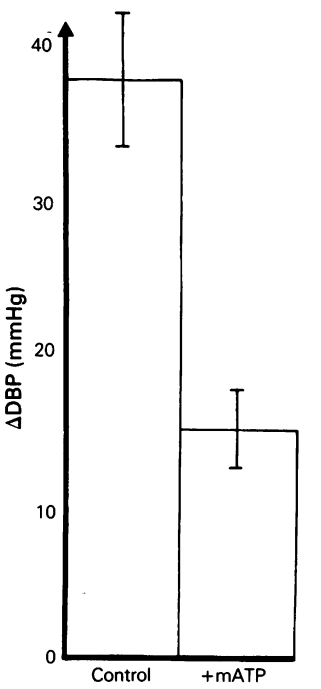

Figure 4 Peak vasopressor responses to sympathetic nerve stimulation (T_6–T_8) for 1 s at 20 Hz were measured as changes in diastolic blood pressure (ΔDBP) (in the absence of α-adrenoceptor antagonists); in control conditions (propranolol 1 mg kg^{-1} and gallamine 10 mg kg^{-1}) and after P_2-purinoceptor desensitizing doses of α,β-methylene ATP (mATP).

α-adrenoceptor antagonists prazosin and rauwolscine together (each 1 mg kg^{-1}) (Figure 3), leaving approximately 60% of the response when stimulating the T_8 region for 1 s at 20 Hz.

In the absence of α-adrenoceptor antagonists, the desensitizing doses of mATP reduced pressor responses to sympathetic nerve stimulation to approximately 40% of control levels (Figure 4).

In the presence of α-adrenoceptor antagonists, subsequent additions of mATP which gave a final cumulative dose of 2.5 mg kg^{-1} completely blocked the pressor responses (Figure 3).

The effect of α,β-methylene ATP on the pressor response to noradrenaline

The effect of the desensitizing dose of mATP was studied on the pressor response to three doses of noradrenaline (NA). Single bolus doses of 0.1, 0.3, and 1 μg kg^{-1} NA gave control dose-dependent pressor responses of 20 ± 5, 36 ± 8, and 58 ± 6 mmHg respectively ($n = 5$, in each case). Within 10 min after the addition of mATP these same three doses of NA gave pressor responses of 22 ± 6, 35 ± 8, and 60 ± 5 mmHg and were not significantly different from the control responses. The sequences of the addition of the NA doses were alternated in each experiment since the blocking effect of mATP on the nerve-mediated pressor responses is time-dependent (Figure 5).

The effect of α,β-methylene ATP on vas deferens responses

The longitudinal tension responses of the vas deferens produced by stimulating the spinal sympathetic outflow and the effects on these of the sequential administration of propranolol, prazosin, rauwolscine and mATP were studied.

Neither propranolol (1 mg kg^{-1}) nor prazosin (1 mg kg^{-1}) affected the monophasic contractions of the vas deferens (Figure 6).

Rauwolscine (1 mg kg^{-1}) significantly increased the responses (Figure 6).

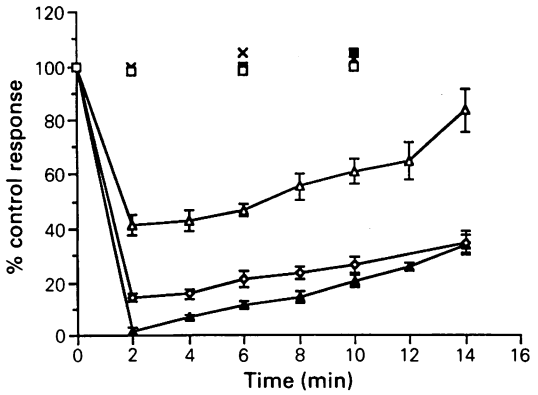

Figure 5 The time-dependent blocking effect of α,β-methylene ATP (mATP) *in vivo* on agonist-induced and nerve-mediated vasopressor responses (T_6–T_8, for 1 s at 20 Hz) and on nerve-mediated motor responses of the vas deferens (T_{13}, for 1 s at 5 Hz). These effects are plotted as % of control response of each preparation at 2 min intervals after the administrations of mATP. The vasopressor responses to noradrenaline (NA, 0.1 μg kg^{-1} (×); 0.3 μg kg^{-1} (■); 1 μg kg^{-1} (□)) were measured immediately after completion of the desensitization schedule for P_2-purinoceptors by mATP. Dose-response curves were completed in each preparation but the sequence of administration was rotated to take into account the short life of mATP *in vivo*. NA responses were measured in the presence of propranolol (1 mg kg^{-1}). Vasopressor responses to sympathetic nerve stimulation were measured both before (△) and after (▲) α-adrenoceptor antagonists had been added. Motor responses of the vas deferens were measured after α-adrenoceptor blockade (◇). These responses were measured in the presence of propranolol (1 mg kg^{-1}) and gallamine (10 mg kg^{-1}). $n = 4$–6

The effects of mATP on the responses of the vas deferens were two fold. The initial effects observed on administration of bolus injections of mATP (0.01–0.1 mg kg^{-1}) were increases in tone of the muscle accompanied by increases in contractions to the sympathetic stimulation. Within 1 min this potentiating effect disappeared and was replaced by an attenuation of the responses (Figure 6).

This blocking effect of mATP was time-dependent. Figure 5 shows the diminishing blocking effect of mATP on various responses with respect to time.

Effects of antagonists on nerve mediated pressor responses in the pithed rabbit

In two pithed rabbits the α-component to stimulation of the spinal outflow was proportionally larger than in the rat (up to 70% reduction in response after the addition of the α-adrenoceptor antagonists rauwolscine (1 mg kg^{-1}) and prazosin (1 mg kg^{-1})).

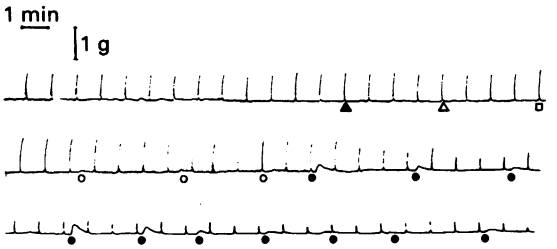

Figure 6 Trace showing the contractile responses obtained by stimulation of the vas deferens of the pithed rat *in situ* (1 cm electrode, T_{13}, 40 V for 1 s at 5 Hz at 1 min intervals). Responses were measured after the sequential administration of propranolol (1 mg kg^{-1}) (▲), prazosin (1 mg kg^{-1}) (△), rauwolscine (1 mg kg^{-1}) (□), α,β-methylene ATP (mATP, 0.01 mg kg^{-1}) (○) and mATP (0.1 mg kg^{-1}) (●).

The same dose schedule for mATP as in the rat produced pressor responses which exhibited tachyphylaxis but caused no detectable change in the α-blocker-resistant pressor response. The stimulation parameters were the same as those used in the rat except that longer pulse trains were employed, in a vain attempt to uncover a purinergic element. The prazosin/rauwolscine-resistant component in this case could only be slightly attenuated by additions of mATP. This might indicate that, for the resistance vessels of the vascular beds stimulated from this particular spinal level, the rabbit has a very small purinergic component in comparison to the rat, which in turn has a relatively small α-adrenergic component.

Discussion

The results suggest that the widespread sympathetic vasopressor response to stimulation of the sympathetic outflow in the rat comprises α-adrenergic and purinergic elements since it can be partly blocked by α-adrenoceptor antagonists or a purinoceptor P_{2x} desensitizing agent but can be completely blocked by a combination.

An attempt was made to analyse any possible purinergic element in two pithed rabbits by use of a similar protocol to that for the rats. There was an α-blocker-resistant component but this was proportionally smaller than in the rat. However, despite varying the pulse number over a wide range, which might have shown up any otherwise hidden, time-related purinergic component, we could not produce any significant blockade with mATP. Tachyphylaxis to the mATP dose schedule occurred but either this did not achieve the same degree of P_{2x}-purinoceptor desensitization as in the rat, or no nerve-mediated

response via this receptor occurs with this form of stimulation in the rabbit.

On its own, mATP significantly attenuated the vasopressor responses to nerve stimulation in the pithed rat, by about 60%. After a combination of α_1- and α_2-adrenoceptor antagonists, the residual response was completely removed by mATP. This blocking effect of mATP was deemed to be specific against P_2-purinoceptors since a similar dose of mATP had no effect on the pressor responses produced by the intravenous administration of noradrenaline. Taken together, this suggests that the response to this particular stimulus (20 pulses at 20 Hz) consists of two additive elements, 40% α-adrenergic (mainly α_1) and 60% purinergic.

mATP attenuates the non-adrenergic component of nerve induced contractions of the vas deferens *in vitro* (Sneddon & Burnstock, 1984). We confirmed this in the pithed rat to verify the selectivity and the time course of blockade of mATP *in vivo*. Whereas a long-lived desensitization can be maintained *in vitro*, in the pithed rat a parallel rapid loss of mATP's effect was clear in both vas deferens and resistance blood vessels, making this a critical consideration when employing mATP as a blocker of putative purinergic transmission. As expected from *in vitro* studies (Brown *et al.*, 1983; McGrath, 1984) the vas deferens 'twitch' to a short train of pulses at high frequency showed no substantial post-junctional α-adrenergic component but this largely purinergic response was under a small degree of autofeedback via α_2-adrenoceptors.

Previously we have shown that the direct pressor response to stimulation of the sympathetic outflow can be only partially blocked by reserpine suggesting that NA is not the only transmitter. However, transmission can be completely blocked by pretreatment with 6-hydroxydopamine (6-OHDA) suggesting that only nerves possessing uptake and accumulation sites for phenylethanolamines are involved (Flavahan *et al.*, 1985). This was consistent with the entire response arising from adrenergic nerves but part of it (the reserpine-resistant element) not requiring NA, as would arise if there was a co-transmitter. However our earlier failure to abolish the response with a combination of mATP and α-adrenoceptor antagonists left open the possibilities of another co-transmitter or of an adrenergic response that was not mediated by α-adrenoceptors. The present results show that this arose because we failed to detect the transience of the blockade with mATP. All 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_2-purinoceptors, respectively.

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