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Short communication

Inhibition of neural ATP release by atrial natriuretic peptide in guinea-pig vas deferens

Bernd Driessen *,[1], Jorge Gonçalves [2]

Pharmakologisches Institut, Albert-Ludwigs-Universität Freiburg, Hermann-Herder-Strasse 5, D-79104 Freiburg i. Br., Germany

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Abstract

Effects of atrial natriuretic peptide (ANP) and 8-bromoguanosine 3′:5′-cyclic monophosphate (8-BrcGMP) on contraction, overflow of tritium (after [^{3}H]noradrenaline labelling) and overflow of ATP elicited by electrical field stimulation (210 pulses/7 Hz) were studied in guinea-pig vas deferens. ANP (1–100 nM) slightly increased contractions, did not alter the overflow of tritium but decreased the overflow of ATP by up to 50%. 8-BrcGMP (3–300 μM) markedly reduced contractions and ATP overflow with no effect on tritium overflow. Contractions were suppressed in the presence of prazosin plus suramin, and evoked overflow of ATP declined to 11%. ANP now gradually increased tritium overflow but again decreased the overflow of ATP. 8-BrcGMP did not change tritium overflow, as before, but increased ATP overflow. The results indicate that ANP inhibits neural release of ATP by a mechanism independent of guanylyl cyclase activation with no major effect on noradrenaline release.

Keywords: Vas deferens, guinea-pig; Noradrenaline release; ATP release; Cotransmission; ANP (atrial natriuretic peptide); 8-Bromoguanosine 3′:5′-cyclic monophosphate

1. Introduction

In several sympathetically innervated tissues atrial natriuretic peptide (ANP) inhibits the action potential evoked release of noradrenaline (see Debinski et al., 1990). Since noradrenaline and ATP are cotransmitters in postganglionic sympathetic neurones (see Burnstock, 1990; Von Kügelgen and Starke, 1991a; Hoyle, 1992), the question arises whether ANP also modulates neural release of ATP. ANP reduced the first, mainly purinergic phase of the neurogenic contraction in guinea-pig (Mutafova-Yambolieva et al., 1993) and rabbit vas deferens (Drewett et al., 1989; Trachte and Drewett, 1994) as well as the isolated purinergic contraction component in guinea-pig vas deferens (Mutafova-Yambolieva et al., 1993). These observations agree with an ANP-mediated inhibition of the purinergic neurotransmission, however, do not provide direct evidence for a decrease of neural ATP release.

We therefore studied the effect of ANP on contraction, [^{3}H]noradrenaline (measured as total tritium) and ATP overflow in the guinea-pig vas deferens in order to investigate whether ANP modulates both noradrenaline and ATP release. Because ANP is known to activate guanylyl cyclase (e.g. Trachte and Drewett, 1994), we tested also the cyclic guanosine nucleotide analogue 8-bromoguanosine 3′:5′-cyclic monophosphate (8-BrcGMP). Sympathetic nerve stimulation elicits an overflow of ATP from guinea-pig vas deferens which is largely non-neural in origin unless precautions are taken (Von Kügelgen and Starke, 1991b; Sperlágh and Vizi, 1992; Driessen et al., 1993; Gonçalves et al., 1995). Thus, some experiments were carried out after blockade of α_1-adrenoceptors (prazosin) and P_2-purinoceptors (suramin) in order to approach, as close as possible, neural release of ATP (Driessen et al., 1993).

* Corresponding author. Tel.: 0049-(0)761-203-5306; fax: 0049-(0)761-203-5318/5311.

[1] Present address: University of California, Veterinary Medical Teaching Hospital, Department of Anesthesiology, Davis, CA 95616-8747, USA.

[2] Present address: Lab. Farmacologia, Fac. Farmácia, Universidade do Porto, Rua Aníbal Cunha, P-4000 Porto, Portugal.

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2. Materials and methods

Methods were as in Driessen et al. (1993), with modifications mentioned in the subsequent brief summary. Male guinea pigs (450–1000 g) were decapitated, the vasa deferentia were removed and cleaned of connective tissue and thereafter preincubated with 0.1 μM [^{3}H](−)-noradrenaline, specific activity 71.7 Ci mmol^{-1}. Each organ was then suspended vertically between two parallel platinum wire electrodes, initial tension 9.8 mN, and superfused for 125 or 185 min at 3 ml min^{-1}. The medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11, ascorbic acid 0.3, and disodium EDTA 0.03; it was saturated with 95% O_2/5% CO_2 and kept at 37°C.

A total of 3 or 5 periods of electrical field stimulation were applied, the first 60 min after the onset of superfusion, interval between beginnings of periods 30 min. Each period consisted of 210 pulses at 7 Hz (0.7 ms pulse width; current strength 45 mA). Only contractions and overflow elicited by the stimulation periods at 90 min and later were evaluated (S_1 to S_4; S_1, S_2). Prazosin plus suramin were administered throughout superfusion. ANP and 8-BrcGMP were added at cumulatively increasing concentrations 25 min before S_2, S_3 and S_4, and tetrodotoxin 1 μM was added 25 min before S_4. In experiments with prazosin plus suramin throughout, ANP was tested at only one concentration per experiment administered 25 min before S_2. At the end of each experiment, preparations were weighed (77 ± 3 mg; n = 72) and solubilized.

The amplitudes of phase I and II of the electrically evoked contractions (see Fig. 1 in Driessen et al., 1993) were evaluated separately.

Tritium was measured in 1 ml aliquots of superfusate samples and solubilized tissues by liquid scintillation spectrometry. Tritium outflow was expressed as fractional rate (min^{-1}) and electrically evoked overflow (total outflow minus basal outflow) as nCi (g tissue)$^{-1}$ or as a percentage of the tritium content of the tissue at the onset of the respective stimulation period. ATP was measured in 0.1 ml aliquots of superfusate samples by means of the luciferin-luciferase technique. Drugs present throughout superfusion were included in blank and calibration curve media. ANP, 8-BrcGMP and tetrodotoxin did not interfere with the assay at the highest concentrations used. Outflow of ATP was expressed as pmol min^{-1} (g tissue)$^{-1}$ and evoked overflow (total minus basal) as pmol (g tissue)$^{-1}$. For further evaluation, ratios were calculated of responses to S_2, S_3 and S_4 (or tritium or ATP outflow immediately before S_2, S_3 and S_4) and responses to S_1 (tritium or ATP outflow immediately before S_1).

Drugs were suramin hexasodium salt (Bayer, Wuppertal, Germany); [*ring*-2,5,6-^{3}H](−)-noradrenaline (DuPont, Dreieich, Germany); prazosin HCl (Pfizer, Sandwich, Kent, UK); rauwolscine HCl (Roth, Karlsruhe, Germany); atrial natriuretic peptide (ANP; rat synthetic); 8-bromoguanosine 3′:5′-cyclic monophosphate sodium salt (8-BrcGMP); tetrodotoxin (Sigma, Deisenhofen, Germany). Drugs were dissolved in distilled water except tetrodotoxin (sodium actetate buffer 0.1 M, pH 4.85) and added to the superfusion fluid reservoir (solvent in controls).

Results are given as arithmetic means ± S.E.M. Differences between means were tested for significance by the Mann-Whitney test. $P < 0.05$ was taken to be statistically significant.

3. Results

3.1. Experiments without prazosin and suramin

As previously described (Driessen et al., 1993), electrical stimulation (210 pulses/7 Hz) in drug-free medium elicited biphasic contractions with an initial twitch (phase I) and a secondary slow phase (phase II) as well as steep tritium and ATP overflow peaks. Absolute values for basal tritium and ATP efflux and for responses to the stimulation period S_1 are given in the legend to Table 1. When tissues were stimulated 4 times in control experiments (solvent before S_2 to S_4), evoked contractions and tritium overflow remained

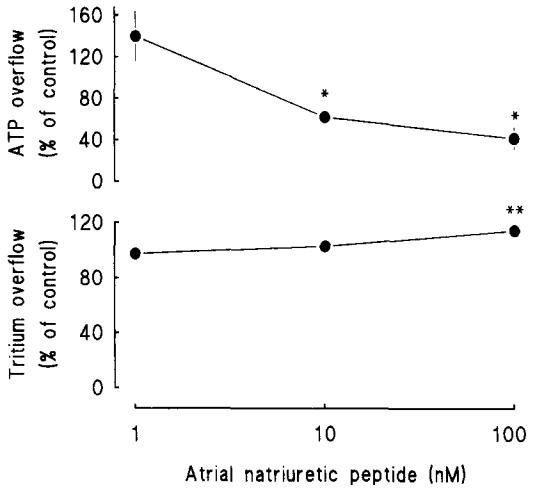

Fig. 1. Effect of atrial natriuretic peptide (ANP) on electrically evoked overflow of tritium and ATP from guinea-pig vas deferens in the presence of prazosin and suramin. Tissues were preincubated with [^{3}H]noradrenaline, then superfused with medium containing prazosin 0.3 μM plus suramin 300 μM and stimulated twice by 210 pulses, 7 Hz (S_1, S_2). ANP 1, 10 or 100 nM was added before S_2. Ordinates show overflow responses (means ± S.E.M. of 5–8 experiments) after addition of ANP as a percentage of controls in which solvent was administered instead of ANP (based on S_2/S_1 ratios; there were 9 controls). Significant differences from corresponding control: * $P < 0.05$; ** $P < 0.01$.

Table 1
Effect of atrial natriuretic peptide (ANP), 8-bromoguanosine 3′:5′-cyclic monophosphate (8-BrcGMP) and tetrodotoxin on electrically evoked contraction, tritium overflow and ATP overflow

Drug added before S_2		Drugs throughout	Responses to electrical stimulation (% of control)				n
			Contraction phase		Tritium overflow	ATP overflow	
			I	II			
Tetrodotoxin	1 μM	–	0 ± 0 [b]	0 ± 0 [b]	0.7 ± 0.2 [b]	0 ± 0 [b]	3
ANP	1 nM	–	108.9 ± 3.3 [a]	111.0 ± 2.2 [a]	102.3 ± 2.6	76.8 ± 13.0	5
ANP	10 nM	–	117.6 ± 3.4 [b]	112.8 ± 4.1 [a]	104.1 ± 3.4	65.5 ± 10.3 [a]	5
ANP	100 nM	–	116.2 ± 3.1 [b]	115.0 ± 4.4 [a]	104.1 ± 5.7	49.5 ± 8.4 [a]	5
8-BrcGMP	3 μM	–	93.7 ± 2.1 [a]	100.7 ± 4.7	89.7 ± 8.9	81.8 ± 12.0	6
8-BrcGMP	30 μM	–	79.5 ± 6.1 [a]	72.7 ± 8.5 [a]	90.2 ± 6.6	62.7 ± 10.2 [a]	6
8-BrcGMP	300 μM	–	36.7 ± 1.8 [b]	38.1 ± 6.2 [b]	80.6 ± 6.1	24.7 ± 3.7 [b]	6
8-BrcGMP	3 μM	Prazosin + suramin	–	–	97.5 ± 3.5	286.3 ± 56.0 [b]	8
8-BrcGMP	30 μM	Prazosin + suramin	–	–	98.5 ± 2.2	211.8 ± 42.0 [a]	8
8-BrcGMP	300 μM	Prazosin + suramin	–	–	108.4 ± 3.8	206.6 ± 57.9 [a]	8

Tissues were preincubated with [^{3}H]noradrenaline, then superfused with medium containing either no drug or prazosin 0.3 μM plus suramin 300 μM and stimulated 4 times by 210 pulses/7 Hz (S_1 to S_4). Atrial natriuretic peptide (ANP) and 8-bromoguanosine 3′:5′-cyclic monophosphate (8-BrcGMP) were added before S_2, S_3 and S_4 at increasing concentrations whereas tetrodotoxin was added before S_4. Responses to electrical stimulation after addition of ANP, 8-BrcGMP or tetrodotoxin are expressed as a percentage of controls in which solvent was administered (based on S_n/S_1 ratios; there were 16 controls with drug-free medium and 6 controls with medium containing prazosin and suramin). Responses to S_1 in drug-free medium ($n = 30$): contraction phase I 31 ± 2 mN; phase II 20 ± 1 mN; overflow of tritium 0.669 ± 0.034% of tissue tritium, corresponding to 159 ± 21 nCi (g tissue)$^{-1}$; of ATP 30.6 ± 5.0 pmol (g tissue)$^{-1}$. The fractional rate of tritium efflux immediately before S_1 was 0.0015 ± 0.0001 min^{-1}; ATP efflux at this time was 0.77 ± 0.08 pmol min^{-1} (g tissue)$^{-1}$. Means ± S.E.M. of n experiments. Significant differences of S_n/S_1 values from corresponding control: [a] $P < 0.05$; [b] $P < 0.01$.

constant with S_n/S_1 ratios close to unity whereas evoked ATP overflow gradually increased from S_1 to S_4, as in a previous study (Driessen et al., 1993), with average S_n/S_1 ratios of 1.4–2.0. Tetrodotoxin 1 μM, when added before S_4, virtually abolished electrically evoked responses (Table 1).

ANP (1–100 nM), when added before S_2 to S_4, slightly increased both phases of the neurogenic contraction without changing evoked tritium overflow, but markedly decreased the evoked overflow of ATP (Table 1). 8-BrcGMP (3–300 μM) reduced contractions, phases I and II, as well as the evoked overflow of ATP in a concentration-dependent manner but did not alter tritium overflow (Table 1). The percentage decrease was similar for both contraction phases and ATP overflow at a given concentration of 8-BrcGMP. Neither drug changed basal tension of the tissue or basal efflux of tritium and ATP.

3.2. Experiments with prazosin and suramin

Electrical stimulation elicited no (33 of 42 experiments) or almost no contraction (9 of 42 experiments) in tissues superfused with medium containing prazosin 0.3 μM and suramin 300 μM. The overflow of tritium elicited at S_1 ($n = 42$) amounted to 0.601 ± 0.027% of tissue tritium, corresponding to 143 ± 8 nCi (g tissue)$^{-1}$, and the fractional rate of tritium efflux immediately before S_1 was 0.0015 ± 0.0001 min^{-1}. When compared to experiments with drug-free medium (cf. legend to Table 1), the evoked overflow of ATP in the presence of prazosin and suramin was greatly reduced by 89% ($P < 0.01$), amounting to 3.4 ± 0.6 pmol (g tissue)$^{-1}$ at S_1 ($n = 42$), whereas the ATP efflux, amounting to 2.58 ± 0.55 pmol min^{-1} (g tissue)$^{-1}$ immediately before S_1, was 3.4-fold increased ($P < 0.05$; see also Driessen et al., 1993; Driessen and Starke, 1994; Gonçalves et al., 1995). When solvent was added before S_2 to S_4, tritium overflow remained constant with S_n/S_1 ratios close to unity but the evoked overflow of ATP again increased from S_1 to S_4 as in previous studies (Driessen et al., 1993; Driessen and Starke, 1994), with average S_n/S_1 ratios of 0.8–2.0.

ANP (1–100 nM), when added before S_2, slightly increased the evoked overflow of tritium, significantly so at 100 nM, but again markedly decreased the evoked overflow of ATP in a concentration-dependent manner (Fig. 1). The percentage decrease of ATP overflow at a given concentration of ANP was similar to the series without antagonists (cf. Table 1). 8-BrcGMP (3–300 μM) did not change the evoked overflow of tritium, as before in the absence of antagonists, but now more than doubled the evoked overflow of ATP at any concentration tested (Table 1). ANP and 8-BrcGMP did not alter basal tension, tritium outflow or ATP outflow in the presence of prazosin and suramin.

4. Discussion

Contractions as well as the overflow of tritium and ATP elicited by 210 pulses/7 Hz in the guinea-pig vas deferens were prevented by tetrodotoxin and, hence, due to neural excitation. The evoked overflow of total

tritium determined in these experiments is an appropriate estimate of neural [^{3}H]noradrenaline release, thus, modulation of tritium overflow can be interpreted as modulation of noradrenaline release (see Driessen et al., 1993; Driessen and Starke, 1994 for discussion).

The present study is the first to demonstrate a decrease by ANP of the electrically evoked overflow of ATP from a sympathetically innervated tissue. It also provides further evidence that in the vas deferens ANP inhibits the purinergic neurotransmission via a prejunctional modulation of ATP release (Drewett et al., 1989; Mutafova-Yambolieva et al., 1993; Trachte and Drewett, 1994). In contrast to rabbit vas deferens (Drewett et al., 1989) as well as the majority of other tissues studied so far (see Debinski et al., 1990), ANP does not seem to reduce noradrenaline release in the guinea-pig vas deferens. It did not change evoked tritium overflow in the absence of other drugs, a result that agrees with the lack of effect on the adrenergic component of the neurogenic contraction (Mutafova-Yambolieva et al., 1993). Contrary to the finding of Mutafova-Yambolieva et al. (1993) in prostatic halves of the guinea-pig vas deferens, contractions (phases I and II) were not diminished but marginally increased by all concentrations of ANP in the present study using the entire organ despite a pronounced reduction of evoked ATP overflow and no change of tritium overflow. We do not know the reason for this discrepancy. In agreement with recent reports (Drewett et al., 1989; Mutafova-Yambolieva et al., 1993) contractions to exogenous ATP (0.3–300 μM) and noradrenaline (0.3–300 μM) were not significantly affected by ANP 100 nM ($n = 3$, Driessen, unpublished observation), thus arguing against a direct postjunctional action of ANP.

As mentioned in the Introduction total ATP overflow measured in the absence of other drugs does not represent neural release of ATP. The 89% decrease caused by prazosin plus suramin in the present study confirms that most of the total overflow in unblocked preparations is postjunctional in origin. Therefore, changes by ANP in postjunctional ATP release (observed in unblocked tissues) must not coincide with changes in neural ATP release but may rather obscure them. However, when non-neural release of ATP was suppressed by means of the antagonists, ANP again reduced evoked ATP overflow and this in a manner similar to its inhibitory action in unblocked preparations. Tritium overflow was only slightly increased by ANP 100 nM. These results indicate that ANP prejunctionally inhibits neural release of ATP without an equal modulatory effect on the release of noradrenaline – with the ensuing basic problem of how this can be explained in the context of the vesicle and exocytosis theory of transmitter release (see Burnstock, 1990; Von Kügelgen and Starke, 1991a).

ANP causes an augmentation of the guanosine 3′:5′-cyclic monophosphate formation in the rabbit vas deferens (Trachte and Drewett, 1994). In order to test whether the reduction of ATP release by ANP was due to activation of guanylyl cyclase we examined the effects of the stable analogue 8-BrcGMP on electrically evoked responses. In accordance with its inhibitory effect on contractile responses of blood vessels to either ATP (e.g. Andriantsitohaina et al., 1995) or noradrenaline (e.g. McMahon and Paul, 1986), 8-BrcGMP markedly and to about the same extent reduced both phases of the neurogenic contraction. Evoked tritium overflow was not changed whilst ATP overflow was decreased by up to about 75%, thus more marked than by ANP. However, 8-BrcGMP might have reduced the non-neural rather than neural release of ATP, possibly by decreasing the contraction response. Therefore, 8-BrcGMP experiments were repeated after suppression of contractions by prazosin plus suramin. In fact, under these conditions evoked overflow of tritium remained unchanged but the inhibition of the ATP overflow was reversed to a marked enhancement. In conjunction with recently published data from rabbit vas deferens (Trachte and Drewett, 1994), these results argue against an involvement of guanylyl cyclase activation in the prejunctional inhibition of neural ATP release by ANP.

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References

Andriantsitohaina, R., G.J. Lagaud, A. Andre, B. Müller and J.C. Stoclet, 1995, Effects of cGMP on calcium handling in ATP-stimulated rat resistance arteries, Am. J. Physiol. 268, H1223.

Burnstock, G., 1990, Co-transmission, Arch. Int. Pharmacodyn. 304, 7.

Debinski, W., O. Kuchel and N.T. Buu, 1990, Atrial natriuretic factor is a new neuromodulatory peptide, Neuroscience 36, 15.

Drewett, J.G., G.J. Trachte and G.R. Marchand, 1989, Atrial natriuretic factor inhibits adrenergic and purinergic neurotransmission in the rabbit isolated vas deferens, J. Pharmacol. Exp. Ther. 248, 135.

Driessen, B. and K. Starke, 1994, Modulation of neural noradrenaline and ATP release by angiotensin II and prostaglandin E_2 in guinea-pig vas deferens, Naunyn-Schmied. Arch. Pharmacol. 350, 618.

Driessen, B., I. Von Kügelgen and K. Starke, 1993, Neural ATP release and its α_2 adrenoceptor-mediated modulation in guinea-pig vas deferens, Naunyn-Schmied. Arch. Pharmacol. 348, 358.

Gonçalves, J., B. Driessen, I. Von Kügelgen and K. Starke, 1995, Comparison of corelease of noradrenaline and ATP evoked by hypogastric nerve stimulation and field stimulation in guinea-pig vas deferens, Naunyn-Schmied. Arch. Pharmacol. 352, 229.

Hoyle, C.H.V., 1992, Transmission: purines, in: Autonomic Neuroeffector Mechanisms, eds. G. Burnstock and C.H.V. Hoyle (Harwood Academic Publishers, Chur, Reading, Paris, Philadelphia, Tokyo, Melbourne) p. 367.

McMahon, E.G. and R.J. Paul, 1986, Effects of forskolin and cyclic nucleotides on isometric force in rat aorta, Am. J. Physiol. 250, C468.

Mutafova-Yambolieva, V.N., K.M. Venkova and L.S. Lasova, 1993, Atrial natriuretic peptide inhibits the purinergic and not the adrenergic component of electrically induced contractile responses in guinea-pig vas deferens, J. Pharmacol. Exp. Ther. 265, 920.

Sperlágh, B. and E.S. Vizi, 1992, Is the neuronal ATP release from guinea-pig vas deferens subject to α_2 adrenoceptor-mediated modulation?, Neuroscience 51, 203.

Trachte, G.J. and J.G. Drewett, 1994, C-Type natriuretic peptide neuromodulates independently of guanylyl cyclase activation, Hypertension 23, 38.

Von Kügelgen, I. and K. Starke, 1991a, Noradrenaline-ATP cotransmission in the sympathetic nervous system, Trends Pharmacol. Sci. 12, 319.

Von Kügelgen, I. and K. Starke, 1991b, Release of noradrenaline and ATP by electrical stimulation and nicotine in guinea-pig vas deferens, Naunyn-Schmied. Arch. Pharmacol. 344, 419.