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Short communication

Involvement of cAMP in modulation of noradrenaline release in the human pulmonary artery

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Summary. After incubation with ^{3}H-noradrenaline, strips of human pulmonary arteries from patients undergoing surgery for lung tumour were superfused with physiological salt solution containing cocaine and corticosterone. Forskolin, AH 21-132 (a cAMP phosphodiesterase inhibitor), 8-Br-cAMP and isoprenaline did not affect the basal tritium efflux from the strips, but produced a concentration-dependent faciliatation of the tritium overflow evoked by transmural electrical stimulation (2 Hz). The facilitatory effect of isoprenaline was potentiated by forskolin which produced a shift to the left of the concentration-response curve of isoprenaline. It is concluded that cAMP plays a role in the modulation of noradrenaline release in the human pulmonary artery and that presynaptic β-adrenoceptors appear to be coupled to an adenylate cyclase in the sympathetic nerve terminals.

Key words: Noradrenaline release – Human pulmonary artery – Forskolin – Cyclic AMP – Presynaptic β-adrenoceptors

Introduction

Human tissues have not yet been used to investigate whether cAMP is involved in the regulation of noradrenaline release from postganglionic sympathetic nerve fibres. Reliable information concerning the role played by cAMP in modulation of noradrenaline release can be obtained by drugs which presumably increase the intraneuronal cAMP concentration. Thus, it has been shown that forskolin (an activator of adenylate cyclase; Metzger and Lindner 1981; Seamon and Daly 1983), membrane-permeable cAMP analogues and phosphodiesterase inhibitors facilitate the electrically evoked noradrenaline release in the guinea-pig vas deferens (Wooten et al. 1973), cat spleen (Cubeddu et al. 1975), rabbit pulmonary artery (Göthert and Hentrich 1984), rat pineal gland (Pelayo et al. 1978) and rat as well as rabbit cerebral cortex slices (Markstein et al. 1984).

It was the aim of the present investigation to study whether the same mechanism is operative in human sympathetic nerve fibres. For this purpose, the effects of 8-Br-cAMP, forskolin and the cAMP phosphodiesterase inhibitor AH 21-132 (Markstein et al. 1984; for chemical structure, see Methods) on the electrically evoked ^{3}H-noradrenaline release in the human pulmonary artery were examined. The sympathetic nerve fibres of this blood vessel are endowed with facilitatory presynaptic β-adrenoceptors (Göthert and Hentrich 1985), and stimulation of β-adrenoceptors is known to express its effect via an activation of adenylate cyclase (Nathanson 1977; Stiles et al. 1984). Hence, we studied whether forskolin increases the facilitatory effect of isoprenaline on noradrenaline release, since forskolin has been shown to potentiate the responses of cAMP generating systems to those agonists (e.g. neurotransmitters or hormones) the effects of which are mediated by an adenylate cyclase (Seamon and Daly 1983).

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Methods

Immediately after pneumonectomy specimens of human pulmonary artery were prepared from lobular or segment arteries (diameter: 3–3.5 mm; thickness of the wall: 0.5–1 mm) in macroscopically tumour-free lung tissue. The patients (either sex, age 35 to 70 years) underwent surgery for lung tumour; they did not suffer from pulmonary or systemic hypertension, and were not treated with drugs acting on adrenoceptors or sympathetic nerve fibres. They received pethidine, promethazine and atropine for premedication, hexobarbital, flunitrazepam or etomidate for induction of anaesthesia, N_2O plus enflurane, halothane or fentanyl for its maintenance, and suxamethonium as well as pancuronium for neuromuscular blockade.

Spirally cut strips (3 × 20 mm) of the segments of pulmonary artery were incubated for 60 min in 1.5 ml physiological salt solution (37° C; composition, see Göthert and Hentrich 1984, 1985) containing (–)-ring-[2,5,6-^{3}H] noradrenaline 0.4 µmol/l (specific activity 43.9–53.5 Ci/mmol), and subsequently mounted vertically at a tension of 2 g between two platinum electrodes; they were superfused with ^{3}H-noradrenaline-free solution of 37° C at a rate of 2 ml/min. The superfusion medium contained cocaine 30 µmol/l and corticosterone 40 µmol/l. Three to five 3-min periods of transmural electrical stimulation (rectangular pulses of 150 mA and 0.3 ms; 2 Hz) were applied to each strip after 93 (S_1), 117 (S_2), 141 (S_3), 165 (S_4) and 189 min (S_5) of superfusion. Basal tritium efflux, stimulation-evoked tritium overflow (above basal efflux; for calculations see Göthert and Hentrich 1984, 1985) and the tritium remaining in each strip at the end of superfusion were determined by liquid scintillation counting.

Means ± SEM for n experiments are given throughout the paper, and Student's t-test was used for comparison of means.

Drugs used. (−)-[ring-2,5,6-^{3}H]-noradrenaline (New England Nuclear, Dreieich, FRG); cocaine hydrochloride (Merck, Darmstadt, FRG); corticosterone, 8-bromoadenosine 3′,5′-cyclic monophosphate (sodium salt; 8-Br-cAMP; Sigma, St. Louis, MO, USA); forskolin (Calbiochem-Behring, La Jolla, CA, USA); cis-6-(p-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methyl-benzo [c] [1,6]-naphthyridine-bis (hydrogenmaleinate) (AH 21-132; Sandoz, Basel, Switzerland); (−)-isoprenaline sulphate (Boehringer, Ingelheim, FRG). The stock solution of forskolin was prepared with dimethylsulfoxide (DMSO; Merck, Darmstadt, FRG), whereas the other drugs were dissolved in water. Dilutions were carried out with physiological salt solution. In the controls for the experiments with forskolin, corresponding concentrations of DMSO (0.014 to 14 mmol/l) were present in the superfusion fluid 9 min before and during S_3, S_4 or S_5. At the concentrations used, DMSO did not alter the basal efflux or electrically evoked overflow of tritium from the vascular strips.

Results

The basal efflux of tritium from strips of the human pulmonary artery preincubated with ^{3}H-noradrenaline was not affected by forskolin, AH 21-132, 8-Br-cAMP (Table 1) and isoprenaline, the latter either in the absence (results not shown) or in the presence of forskolin (Table 1). The electrically evoked overflow of tritium was facilitated by forskolin, AH 21-132, 8-Br-cAMP (Fig. 1A) and isoprenaline (Fig. 1B) in a concentration-dependent manner. At the highest concentration investigated the drugs increased the evoked overflow by about 70% − 100%. Preliminary experiments with constant concentrations of the drugs, present from 9 min before S_3 or S_4 until 12 min after the subsequent stimulation period (S_4 or S_5), revealed a constant degree of facilitation (i.e. no time-dependent alteration; results not shown).

In the presence or forskolin 0.3 μmol/l, which by itself produced only a rather small increase in evoked ^{3}H overflow (by about 35%, see Fig. 1A), the concentration-response curve of isoprenaline for its facilitatory effect on evoked overflow was shifted to the left (Fig. 1B). Isoprenaline 0.01 μmol/l, which did not facilitate the evoked ^{3}H overflow in the absence of forskolin, produced a clear-cut increase in evoked overflow when forskolin 0.3 μmol/l was present (Fig. 1B).

Table 1. Effects of forskolin, AH 21-132, 8-Br-cAMP and isoprenaline on the basal tritium efflux from strips of the human pulmonary artery superfused with physiological salt solution containing cocaine and corticosterone. For further details see legend to Fig. 1

Drug	μmol/l	Basal ^{3}H efflux (% of controls)
Forskolin	0.01	102 ± 1
	0.1	100 ± 1
	1	97 ± 1
	10	93 ± 3
AH 21-132	0.1	96 ± 1
	1	96 ± 1
	10	95 ± 3
	30	108 ± 3
8-Br-cAMP	1	95 ± 3
	10	98 ± 2
	100	98 ± 3
	330	95 ± 3
Isoprenaline[a]	0.001	97 ± 4
	0.01	102 ± 5
	0.1	104 ± 5
	1	108 ± 7

[a] In the presence of forskolin 0.3 μmol/l from 24 min before t_2 until the end of the experiments

Basal efflux refers to the four 3-min collection periods (t_2 to t_5) immediately before the second to fifth periods of transmural electrical stimulation (S_2 to S_5). The ratios of the basal efflux during t_3, t_4 or t_5 to that during t_2 are given. They are expressed as percentages of the ratios in the respective control experiments. In the controls belonging to the experiments with AH 21-132 and 8-Br-cAMP, the ^{3}H efflux during t_2 was 2.72 ± 0.46 nCi, corresponding to a fractional rate of efflux of 0.0019 ± 0.0001 per min (related to tissue tritium), and the ratios t_3/t_2, t_4/t_2 and t_5/t_2 were 0.89 ± 0.01, 0.81 ± 0.01 and 0.74 ± 0.02, respectively. Each concentration of the drugs was present 6 min before and during t_3, t_4 or t_5. Means ± SEM of 5 − 7 experiments

Discussion

It was found in the present investigation that forskolin which presumably increases intraneuronal cAMP by direct activation of the catalytic subunit of adenylate cyclase (for review, see Seamon and Daly 1983) did not affect the basal tritium efflux but increased the electrically evoked overflow of tritium from the human pulmonary artery preincubated with ^{3}H-noradrenaline. This finding is in agreement with results obtained in the rabbit pulmonary artery (Göthert and Hentrich 1984) in which, however, the facilitatory effect was apparent only at a ten times higher concentration of the drug than in the human tissue. The electrically evoked tritium overflow from the human pulmonary artery is Ca^{2+}-dependent and abolished by tetrodotoxin (unpublished results). Due to the blockade of the neuronal and extraneuronal uptake mechanisms by cocaine and corticosterone, respectively, it consists almost exclusively of ^{3}H-noradrenaline (about 90% of the ^{3}H-compounds; Göthert and Hentrich 1985). Hence, evoked tritium overflow reflects action potential-induced noradrenaline release from the sympathetic nerve fibres.

AH 21-132 which also facilitated the evoked ^{3}H-noradrenaline release without affecting basal efflux is an inhibitor of cAMP phosphodiesterase (devoid of an antagonistic effect on adenosine receptors; Markstein et al. 1984), and, thus, may increase intraneuronal cAMP. Facilitatory effects of phosphodiesterase inhibitors on the electrically evoked noradrenaline release were also observed in the guinea-pig vas deferens (Wooten et al. 1973; Cubeddu 1975; Stjärne et al. 1979), cat spleen (Celuch et al. 1978) and rabbit pulmonary artery (Göthert and Hentrich 1984). Furthermore, in agreement with previous results in animal tissues (references see above), 8-Br-cAMP, a membrane-permeable cAMP analogue, also facilitated the electrically evoked ^{3}H-noradrenaline release in the human pulmonary artery, without influencing basal tritum efflux.

The results obtained with pharmacological tools which presumably increase intraneuronal cAMP content suggest that the human sympathetic nerve fibres are endowed with a cAMP generating and degrading enzyme system, and that

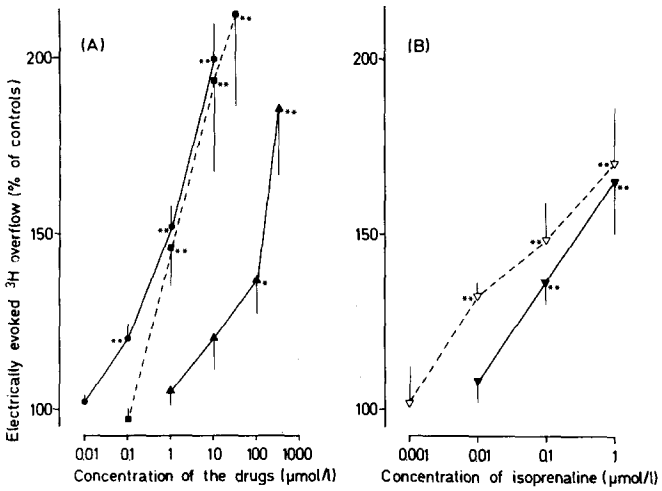

Fig. 1. Effects of forskolin, AH 21-132, 8-Br-cAMP (**A**) and isoprenaline (**B**; in the absence or presence of forskolin) on evoked tritium overflow from human pulmonary artery. Preincubation of the vascular strips with ^{3}H-noradrenaline and superfusion with ^{3}H-noradrenaline-free solution containing cocaine 30 μmol/l and corticosterone 40 μmol/l. Three or five periods of transmural electrical stimulation (S_1 to S_3 or S_5) were applied to each strip at intervals of 24 min. The ratios of the ^{3}H overflow evoked by S_3, S_4 and S_5 to that evoked by S_2 are given. All ratios are expressed as percentage of the ratios in the respective control experiments. In the controls belonging to the experiments with AH 21-132 and 8-Br-cAMP, the ^{3}H overflow evoked by S_2 amounted to 12.54 ± 4.04 nCi (corresponding to 2.50 ± 0.54% of tissue tritium), and the ratios S_3/S_2, S_4/S_2 and S_5/S_2 were 0.89 ± 0.02, 0.81 ± 0.02 and 0.78 ± 0.04, respectively. **A** Each concentration of forskolin (●), AH 21-132 (■) or 8-Br-cAMP (▲) was present 9 min before and during the stimulation period S_3, S_4 or S_5. **B** Each concentration of isoprenaline was present 9 min before and during S_3, S_4 or S_5; effect of isoprenaline without forskolin: ▼; effect of isoprenaline in the presence of forskolin 0.3 μmol/l from 3 min before S_1 until the end of the experiments: ▽. Means ± SEM of 5–7 experiments. * $P < 0.01$, ** $P < 0.005$ (compared to the corresponding controls)

cAMP appears to play a role in the regulation of action potential-induced noradrenaline release. However, in view of the lack of effects on basal tritium efflux, it is probably not crucially involved in the initiation of the release mechanism itself.

Furthermore our experiments revealed that the drugs presumably increasing intraneuronal cAMP content mimic the effect of isoprenaline which activates facilitatory presynaptic β_2-adrenoceptors on the sympathetic nerve fibres of the human pulmonary artery (Göthert and Hentrich 1985). The suggestion derived from this observation, namely that the presynaptic β-adrenoceptors are coupled to an adenylate cyclase, is supported by the interaction experiments of isoprenaline with forskolin: this drug produced a shift to the left of the concentration-response curve of isoprenaline for its facilitatory effect on ^{3}H-noradrenaline release. As already stated above, forskolin is known to potentiate the responses of cAMP generating systems to such agonists, the effects of which are mediated by an adenylate cyclase (Seamon and Daly 1983).

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