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Role of cyclic AMP in the prejunctional α_2-adrenoceptor modulation of noradrenaline release from the rat tail artery

B. Bucher, L. Pain, J.-C. Stoclet, and P. Illes[1]

Laboratoire de Pharmacologie Cellulaire et Moléculaire, C. N. R. S. URA 600, 74, route du Rhin, F-67400 Illkirch, France
[1] Pharmakologisches Institut, Universität Freiburg, Hermann-Herder-Strasse 5, W-7800 Freiburg, Federal Republic of Germany

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Summary. Experiments were designed to evaluate the effect of cyclic AMP on the electrically-induced release of noradrenaline from vascular sympathetic nerve terminals. The possible implication of the inhibition of adenylate cyclase in the negative feed-back control by prejunctional α_2-adrenoceptors of neurotransmitter release was also investigated. Rat isolated tail arteries were preincubated with [^{3}H]-noradrenaline; the preparations were subsequently perfused/superfused with [^{3}H]-noradrenaline-free medium and their perivascular nerves were field stimulated with 24 pulses at 0.4 Hz (0.3 ms, 200 mA). 2 compounds known to enhance the intracellular concentration of cyclic AMP, namely the membrane permeant analogue 8-Br-cAMP (10–300 μmol/l) and forskolin (0.3–10 μmol/l), an activator of adenylate cyclase, concentration-dependently enhanced the stimulation-evoked tritium overflow. The 1,9-dideoxy derivative of forskolin, which does not stimulate adenylate cyclase, was ineffective. Exposure to the cyclic AMP phosphodiesterase inhibitor rolipram 30 μmol/l produced a moderate increase (about 20%) in tritium overflow. However, in the presence of rolipram the facilitatory effect of forskolin was significantly more pronounced than in its absence. Whereas 8-Br-cAMP produced a slight concentration-dependent enhancement of the stimulation-induced vasoconstriction, forskolin and rolipram depressed it.

The α_2-adrenoceptor agonist B-HT 933 (3–30 μmol/l) concentration-dependently inhibited the tritium overflow. The effect of B-HT 933 30 μmol/l was slightly, but significantly reduced in the presence of 8-Br-cAMP 100 and 300 μmol/l, but was not changed in the presence of forskolin 3 μmol/l. The facilitatory effect of rauwolscine 1 μmol/l was enhanced in the presence of 8-Br-cAMP 100 μmol/l. During perfusion with 8-Br-cAMP 100 μmol/l, the current strength and frequency were decreased to 150 mA and 0.2 Hz, respectively in order to obtain similar amounts of tritium overflow to those observed in the absence of the cyclic AMP analogue with the initial stimulation parameters. Under these conditions, the inhibition of the overflow by B-HT 933 30 μmol/l and the facilitation by the α_2-adrenoceptor antagonist rauwolscine 1 μmol/l were unaltered as compared to controls under initial stimulation conditions.

It is concluded that, in the rat tail artery, the terminals of perivascular sympathetic nerves are endowed with an adenylate cyclase system. Cyclic AMP is able to modulate noradrenaline release, but does not appear to play a role in the initiation of the release process itself. In addition, the results do not support the hypothesis that prejunctional α_2-adrenoceptors depress noradrenaline release through the inhibition of adenylate cyclase.

Key words: Noradrenaline release – Forskolin – Cyclic AMP – B-HT 933 – Rauwolscine – Prejunctional α_2-adrenoceptors – Rat tail artery

Send offprint requests to B. Bucher at the above address

Introduction

The existence of prejunctionally located α_2-adrenoceptors mediating feedback inhibition of noradrenaline release from peripheral and central noradrenergic nerve terminals is well documented (see Langer 1981; Starke 1981, 1987). Neurotransmitter release is a process dependent on the level of free axoplasmic calcium (Blaustein 1979). Thus prejunctional inhibition of transmitter release results most probably from a direct or indirect reduction of Ca^{2+} influx into the nerve terminals (Illes 1986). However, the biochemical mechanisms underlying this modulatory process are still unclear. One of the possibilities is that prejunctional receptors are coupled either positively or negatively to adenylate cyclase (Rodbell 1980) or to ion channels (see Brown and Birnbaumer 1988) through guanine nucleotide regulatory (G) proteins. The transmembrane signalling pathways through G proteins are rather complex (see Neer and Clapham 1988) since it has recently been shown that one G protein

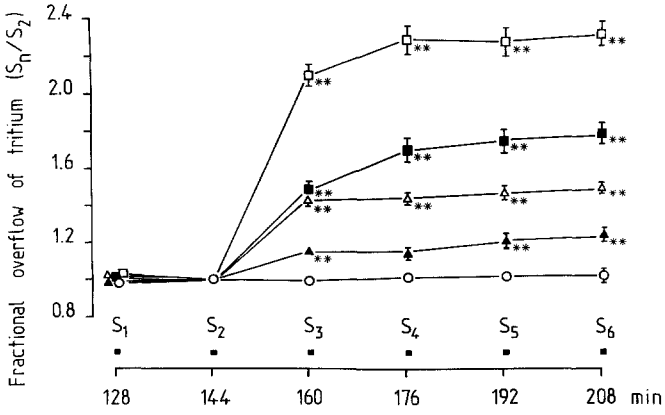

Fig. 1. Time-course of the effects of 8-Br-cAMP, forskolin and rolipram on the stimulation-evoked tritium overflow in rat tail arteries pre-incubated with [^{3}H]-noradrenaline. 6 periods (S_1-S_6) of field stimulation were delivered at intervals of 16 min (24 pulses at 0.4 Hz, 0.3 ms, 200 mA). The solvent (distilled water: ○), 8-Br-cAMP 100 μmol/l (■) and 300 μmol/l (□), forskolin 3 μmol/l (△) and rolipram 30 μmol/l (▲) were added 10 min before S_3 and maintained in the medium for the duration of the experiment. The tritium overflow evoked by S_2 (in the absence of drugs) was $0.208 \pm 0.008\%$ of tissue tritium ($n = 38$; all appropriate experiments pooled). The effects of the drugs are presented as the ratios of tritium overflow evoked by any stimulation period (S_n) over that evoked by S_2. Means ± SEM from 6–10 arteries. Significant differences from each of the experimental groups vs the group with solvent: ** $P < 0.01$

may regulate more than one effector function, for example adenylate cyclase and dihydropyridine-sensitive Ca^{2+} channels (Mattera et al. 1989).

In several tissues it has been observed that α_2-adrenoceptor activation results in a reduction of the intracellular adenosine 3′,5′-cyclic monophosphate (cyclic AMP) concentration by inhibiting adenylate cyclase (Jakobs et al. 1981; Exton 1982). It has been, therefore, suggested that prejunctional α_2-adrenoceptor-mediated inhibition of noradrenaline release is due to a decreased adenylate cyclase activity which in turn decreases the availability of Ca^{2+} for the exocytotic release of the neurotransmitter (see Mulder and Schoffelmeer 1985; Illes 1986). Experiments performed in brain slices support this hypothesis (Schoffelmeer and Mulder 1983; Schoffelmeer et al. 1986), while experiments performed in sympathetically innervated peripheral organs refute it (cat spleen: Cubeddu et al. 1975; guinea pig ileum myenteric-plexus: Alberts et al. 1985; mouse atrium: Johnston et al. 1987).

In view of the controversial results relating to central and peripheral noradrenergic neurones, we decided to meticulously reinvestigate the involvement of cyclic AMP in the inhibitory function of α_2-adrenoceptors in a vascular preparation, the rat tail artery. We measured the nerve stimulation-evoked release of [^{3}H]-noradrenaline and its modulation by α_2-adrenoceptor ligands both under control conditions and after increasing the axoplasmic levels of cyclic AMP with various pharmacological tools (application of a membrane permeating cyclic AMP analogue, activation of the adenylate cyclase by forskolin, and blockade of the degradation of cyclic AMP by phosphodiesterase). Moreover a constant biophase concentration of noradrenaline both before and after such manipulations was ensured by varying the stimulation parameters. In contrast to some previous reports (Cubeddu et al. 1975; Alberts et al. 1985) both α_2-adrenoceptor agonists and antagonists were used.

Material and methods

Measurements of [^{3}H]-noradrenaline overflow and vasoconstriction. Male Wistar rats (12 weeks old) were killed by cervical dislocation. A segment of the ventral tail artery about 2–2.5 cm long was dissected free (Bucher et al. 1987; Illes et al. 1987). It was kept in aerated (95% O_2; 5% CO_2) medium which contained (mmol/l): NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 0.9, $NaHCO_3$ 25, glucose 11, ascorbic acid 0.3 and Na_2EDTA 0.03.

The artery was cannulated at the proximal end and tied off at the distal end. A hole was made through the artery wall just below the tie. The segment was then preincubated for 1 h at 37° C in 1.5 ml of the above solution containing 2.2 μmol/l (−)-[^{3}H]-noradrenaline (specific activity 4.4 Ci/mmol). The artery was then washed 3 times with 20 ml of [^{3}H]-noradrenaline-free medium and suspended vertically, distal end uppermost, between 2 platinum wire electrodes and perfused at a rate of 2.2 ml/min with the same medium using a peristaltic pump. Throughout the duration of the perfusion this solution contained cocaine 10 μmol/l in order to block the re-uptake of released [^{3}H]-noradrenaline. After having passed through the lumen, the perfusate was allowed to superfuse the advential surface of the vessel. Perfusion pressure was measured by using a transducer and was recorded on a pen recorder. Vasoconstriction was determined as an increase in perfusion pressure which reflected changes in the resistance to flow.

The first stimulation period was applied after 96 min of perfusion and others followed at intervals of 16 min. Collection of the perfusate/superfusate started after 124 min of perfusion in 1, 2 or 6 min fractions. The stimulation period beginning at 128 min was termed S_1 and subsequent ones S_2-S_n. In most of the experiments the two first stimulation periods (i.e. S_1 and S_2) consisted of 24 monophasic rectangular pulses of 0.3 ms width at supramaximal strength (200 mA) delivered at 0.4 Hz. S_1 and S_2 served as a control of any drug effect on stimulation-evoked tritium overflow. The drugs were infused with a syringe pump into the perfusion stream at a rate of 17 μl/min for 10 min before S_3 and maintained until the end of the experiment.

Preliminary pilot experiments had shown that these parameters of field stimulation excited nerves exclusively, since the stimulation-evoked tritium overflow from arteries preloaded with [^{3}H]-noradrenaline was almost abolished by tetrodotoxin 0.5 μmol/l ($n = 6$) or in a Ca^{2+}-deficient medium ($n = 6$). Moreover α-adrenoceptor antagonists (α_1: prazosin 0.1 μmol/l; α_2: rauwolscine 3 μmol/l) reduced the contractile response to field stimulation by about 75% ($n = 6$). A subsequent desensitization by α,β-methylene ATP 10 μmol/l nearly abolished the residual contractions which were resistant to α-adrenoceptor blockade, confirming thereby that both noradrenaline and ATP are involved in sympathetic neurotransmission in the rat tail artery (Sneddon and Burnstock 1984; Bao et al. 1989).

When the effect of a second release-modulating drug, i.e. B-HT 933 is evaluated, it is important to obtain similar reference values despite the presence of the first release-modulating compound, i.e. 8-Br-cAMP or forskolin. In other words the biophase concentration of noradrenaline has to be approximately the same in the presence of the modulating compound as in its absence. Thus, the stimulation parameters were varied in order to adjust the amount of stimulation-evoked tritium overflow in the presence of the first modulating drug to what without it (S_3/S_2 close to 1). The electrical stimulation consisted of a train of 12 monophasic pulses of 0.3 ms width at 150 mA current strength delivered at 0.2 Hz instead of 24 pulses at

Table 1. Effects of drugs on the basal outflow of tritium from tail arteries pre-incubated with [^{3}H]-noradrenaline and subsequently perfused/superfused with [^{3}H]-noradrenaline-free medium containing cocaine 10 μmol/l. The values of b_n represent the fractional rate of tritium outflow in the 2 min before the corresponding stimulation period S_n. The ratios of the basal outflow during b_3, b_4, b_5 or b_6 to that during b_2 were calculated

Drug present from S_3 onwards (μmol/l)	n	b_3/b_2	b_4/b_2	b_5/b_2	b_6/b_2
Control	8	0.968 ± 0.009	0.910 ± 0.010	0.898 ± 0.016	0.878 ± 0.027
8-Br-cAMP 100	6	0.982 ± 0.019	0.990 ± 0.027*	0.972 ± 0.018*	0.962 ± 0.022
8-Br-cAMP 300	8	1.055 ± 0.013**	1.051 ± 0.023**	0.998 ± 0.021**	0.977 ± 0.016*
Forskolin 3	10	0.998 ± 0.017**	0.952 ± 0.010**	0.928 ± 0.012*	0.918 ± 0.017*
Rolipram 30	6	1.127 ± 0.026**	1.050 ± 0.020**	0.988 ± 0.008**	0.933 ± 0.026

Means ± SEM of *n* experiments are indicated. Significant differences from drug-free control experiments: * $P < 0.05$; ** $P < 0.01$

0.4 Hz, 0.3 ms, 200 mA used previously. This modified stimulation protocol was used in some experiments, when 8-Br-cAMP 100 μmol/l was present from S_3 onwards. In other experiments, arteries were only stimulated four times. This was possible because it has been shown that a stable evoked overflow was reached after the second stimulation period in the presence of either 8-Br-cAMP 100 μmol/l or rolipram 30 μmol/l and that no significant changes were observed during those following. Rolipram or 8-Br-cAMP were added 10 min prior to S_1 and maintained for the duration of the whole experiment. The second compound, rauwolscine 1 μmol/l or forskolin 3 μmol/l was given 10 min prior to S_3 and also maintained for the remainder of the experiment. After the end of perfusion, the arteries were solubilized in 1 ml Soluene 100 (Packard Instrument, Paris, France). Tritium was measured by liquid scintillation spectrometry.

Tritium outflow was calculated as a fraction of the amount of tritium present in the tissue at the onset of the respective collection period (fractional rate of outflow). For evaluation of stimulation-evoked tritium overflow, the difference was calculated between the overall tritium outflow during stimulation plus the following 4 min and the estimated basal outflow. The latter was assumed to change linearly from the collection period before the beginning of stimulation to that 5 min after the onset of stimulation. The evoked tritium overflow was calculated as percentage of the amount of tissue tritium at the onset of the respective stimulation period.

Drug effects on the basal outflow were quantified in the following way: the ratios of the fractional rate of outflow (b_n) in the fraction collected immediately before the onset of the stimulation periods in the presence of drugs (S_3-S_6) over the fractional rate of outflow in the fraction immediately before the onset of the last stimulation period preceding the application of any drug (S_2), were calculated (b_n/b_2). In order to quantify effects of drugs on the evoked overflow, the ratio of the overflow evoked by the stimulation period in the presence of the drug, at its maximal effect (S_3-S_5), over the last evoked overflow preceding application of the drug (S_2 or S_4) was determined.

Drugs. The following drugs were used: (−)-noradrenaline hydrochloride, 8-bromoadenosine 3′,5′-cyclic monophosphate (sodium salt; 8-Br-cAMP), forskolin, α,β-methylene ATP (lithium salt), tetrodotoxin (Sigma, L'Isle d'Abeau Chesnes, France); 1,9-dideoxy-forskolin (Calbiochem, La Jolla, CA, USA); cocaine hydrochloride (Coopération Pharmaceutique Française, Nancy, France); B-HT 933 (azepexole, 2-amino-6-ethyl-5,6,7,8-tetrahydro-4H-oxazolo-[4,5-d]-azepine) dihydrochloride (kindly donated by Boehringer Ingelheim, FRG); rauwolscine hydrochloride (Roth, Karlsruhe, FRG); rolipram (kindly donated by Schering, Berlin, FRG).

(−)-[Ring-2,5,6-^{3}H]-noradrenaline, specific activity 40.8–43.7 Ci/mmol (New England Nuclear, Dreieich, FRG) was diluted with unlabelled (−)-noradrenaline hydrochloride in order to obtain a specific activity of 4.4 Ci/mmol. Drugs were dissolved in water. Forskolin and 1,9-dideoxy-forskolin were initially dissolved in ethanol and rolipram in dimethylsulfoxide (DMSO). Further dilutions were with distilled water. Preliminary experiments showed that at the concentration used, neither ethanol nor DMSO (except in the case of 1,9-dideoxy-forskolin; final ethanol concentration 1%) altered the basal outflow or the electrically evoked overflow of tritium.

Statistics. Results are given as means ± SEM. The non-parametric U-test of Mann-Whitney was used for the comparison of mean values, if the Kruskal-Wallis analysis indicated a significant difference between group means. A probability level of 0.05 or less was considered significant.

Results

Time-course of the effects of 8-Br-cAMP, forskolin and rolipram on the electrically-evoked tritium overflow and vasoconstriction

When the time-course of the effects of 8-Br-cAMP 100 and 300 μmol/l, forskolin 3 μmol/l and rolipram 30 μmol/l were studied on the stimulation-evoked tritium overflow and vasoconstriction, the tail arteries were stimulated 6 times. The tritium overflow and peak vasoconstriction evoked by S_2 (before the application of drugs) amounted respectively to 0.208 ± 0.008% of tissue tritium and 98.9 ± 4.5 mm Hg ($n = 38$; all appropriate experiments pooled). As shown in Fig. 1, under control conditions the stimulation-evoked overflow did not change from S_1 to S_2; the ratios were all rather similar and close to unity. When rolipram, forskolin or 8-Br-cAMP were added to the perfusion medium 10 min before S_3, the stimulation-evoked tritium overflow was significantly increased to a new steady-state. In control experiments, the fractional rate of basal tritium outflow declined with time (Table 1). The basal rate of tritium outflow was however slightly increased by 8-Br-cAMP 300 μmol/l, forskolin 3 μmol/l and rolipram 30 μmol/l.

The constrictor responses to electrical stimulation were also affected by the drugs investigated. The extent of facilitation or inhibition reached its maximum at S_3 and remained constant thereafter. The control S_6/S_2 ratio (0.97 ± 0.05; $n = 8$) was significantly increased by both 100 and 300 μmol/l 8-Br-cAMP (to 1.17 ± 0.05; $n = 6$ and 1.35 ± 0.06; $n = 8$, respectively; $P < 0.05$ for each). In contrast, forskolin 3 μmol/l and rolipram 30 μmol/l markedly and significantly attenuated the S_6/S_2 ratio

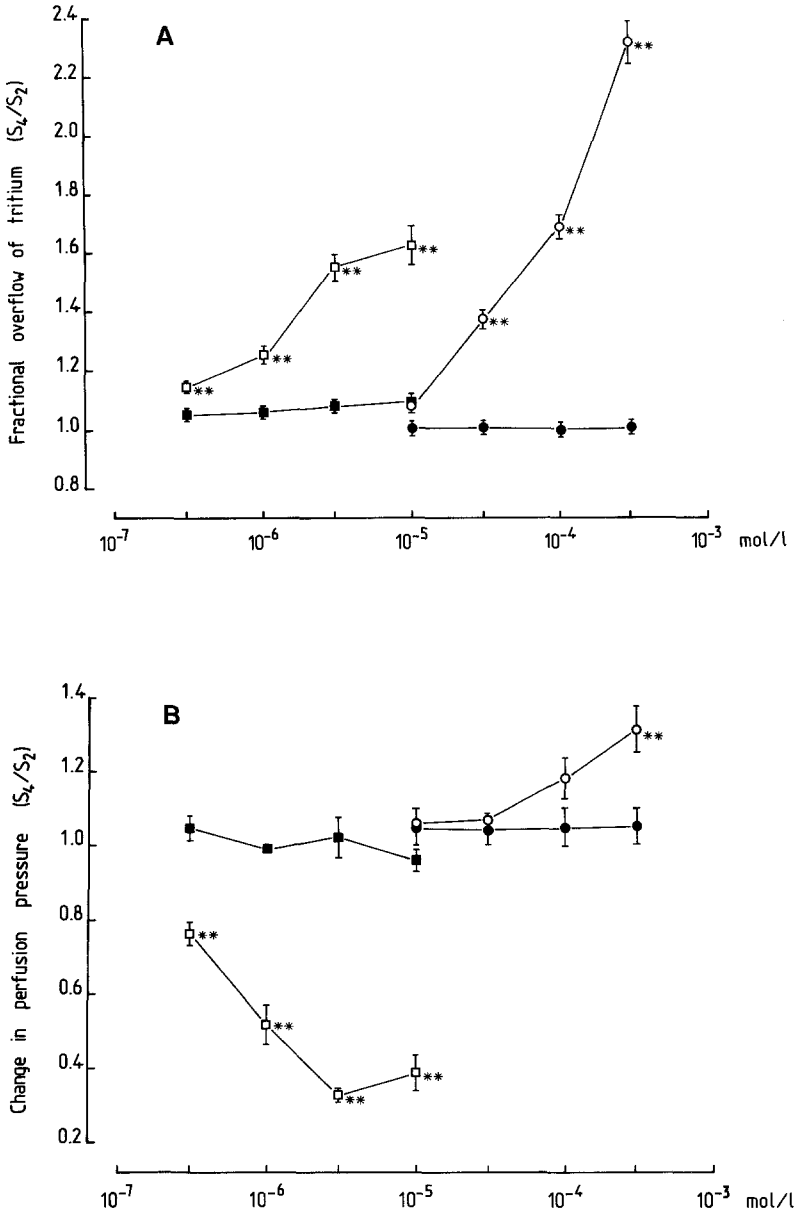

Fig. 2A, B. Concentration-dependent effects of 8-Br-cAMP and forskolin on the electrically-evoked overflow of tritium (**A**) and on the change in perfusion pressure (**B**) in rat tail arteries pre-incubated with [^{3}H]-noradrenaline. 4 periods ($S_1 - S_4$) of field stimulation were delivered at intervals of 16 min (24 pulses at 0.4 Hz, 0.3 ms, 200 mA). Each concentration of 8-Br-cAMP (○) or its solvent (●) and forskolin (□) or its solvent (■) was added 10 min before S_3 and maintained in the medium for the duration of the experiment. The tritium overflow and peak vasoconstriction evoked by S_2 (in the absence of drugs) was respectively $0.233 \pm 0.007\%$ of tissue tritium and 102.9 ± 2.4 mm Hg ($n = 101$; all appropriate experiments pooled). The effects of the drugs are presented as the ratios of tritium overflow or change in perfusion pressure evoked by S_4 over that evoked by S_2. Mean ± SEM from 5–15 arteries. Significant differences from each of the experimental groups vs corresponding groups with solvent: ** $P < 0.01$

when compared to the control value (0.55 ± 0.06; $n = 6$ and 0.67 ± 0.07; $n = 6$, respectively; $P < 0.05$ for each).

Effects of 8-Br-cAMP, forskolin and rolipram on the electrically-evoked tritium overflow and vasoconstriction

Concentration-response curves to 8-Br-cAMP and forskolin, were constructed on the basis of the S_4/S_2 ratios. Four 1 min periods ($S_1 - S_4$) of electrical stimulation were applied; the compounds were added 10 min before S_3 and maintained in the perfusion/superfusion medium for the duration of the experiment. The electrically-evoked tritium overflow was increased in a concentration-dependent manner by the addition of 8-Br-cAMP 30–300 μmol/l and forskolin 0.3–10 μmol/l when compared to experiments with the respective solvents (Fig. 2A). Although forskolin was more potent than 8-Br-cAMP, the maximum effect (S_4/S_2) obtained with 10 μmol/l forskolin (1.62 ± 0.06; $n = 8$; $P < 0.01$) was lower than the maximum effect obtained with 8-Br-cAMP 300 μmol/l (2.31 ± 0.07; $n = 15$; $P < 0.01$).

Effects on the stimulation-induced vasoconstriction were also analysed. As shown in Fig. 2B, 8-Br-cAMP concentration-dependently increased the constrictor responses. This effect was significant at 300 μmol/l, but far less pronounced than the effect observed on the tritium overflow. In contrast, forskolin caused a marked concentration-dependent decrease in stimulation-evoked vasoconstriction. This inhibitory effect was significant at 0.3 μmol/l and maximal at 3 μmol/l. The solvents for these two compounds did not significantly influence vasoconstrictor responses.

Since rolipram 30 μmol/l, only slightly increased the electrically-evoked overflow (see Fig. 1), we investigated the effect of this high concentration in combination with

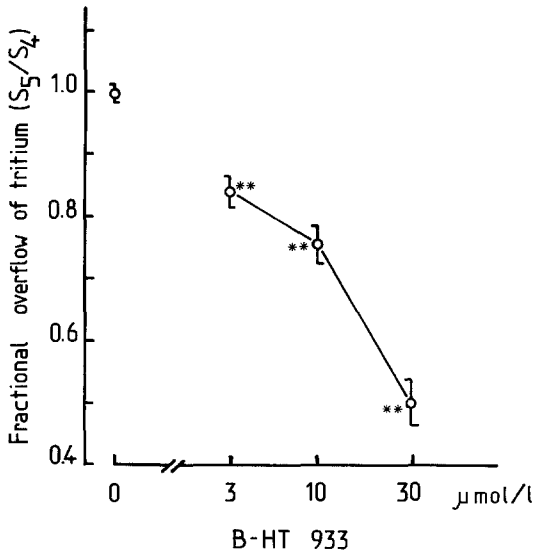

Fig. 3. Concentration-dependent effect of B-HT 933 on the electrically-evoked overflow of tritium in rat tail arteries preincubated with [^{3}H]-noradrenaline. 6 periods (S_1-S_6) of field stimulation were delivered at intervals of 16 min (24 pulses at 0.4 Hz, 0.3 ms, 200 mA). B-HT 933 or its solvent was added 10 min before S_5 and maintained in the medium for the duration of the experiment. The tritium overflow evoked by S_4 (before the application of B-HT 933) was 0.206 ± 0.008% of tissue tritium ($n = 31$; all appropriate experiments pooled). The effect of the drug is presented as the ratios of tritium overflow evoked at S_5 over that evoked at S_4. Means ± SEM from 7–8 arteries. Significant differences from each of the experimental groups vs the corresponding group with solvent: ** $P < 0.01$

forskolin 3 μmol/l. The facilitatory effect of forskolin on the electrically-evoked release of tritium was significantly more pronounced in the presence than in the absence of the phosphodiesterase inhibitor; the respective S_4/S_2 ratios were 1.61 ± 0.05; $n = 7$ and 1.47 ± 0.02; $n = 7$; $P < 0.05$. Likewise, when forskolin 3 μmol/l was added in combination with rolipram 30 μmol/l the attenuation of the stimulation-induced vasoconstriction (S_4/S_2) was significantly more pronounced (0.35 ± 0.03; $n = 7$; $P < 0.05$) than in the absence of the phosphodiesterase inhibitor (0.48 ± 0.04; $n = 7$).

We investigated also the effect of the 1,9-dideoxy derivative of forskolin (DDF) which does not activate adenylate cyclase. In the presence of DDF 10 μmol/l, the stimulation-evoked tritium overflow was almost identical with the control value (S_4/S_2: 1.27 ± 0.02; $n = 6$ and 1.19 ± 0.05; $n = 6$ respectively; $P > 0.05$). The slight enhancement of the overflow was due to the high concentration of ethanol, which was used as a solvent of DDF. Similarly, unlike forskolin, DDF failed to decrease the stimulation-induced vasoconstriction. The S_4/S_2 ratio did not change when compared with the corresponding controls (1.05 ± 0.10, $n = 6$ and 1.07 ± 0.05, $n = 6$ respectively; $P > 0.05$).

Effect of B-HT 933 on the electrically-evoked tritium overflow and vasoconstriction

When concentration-response curves of B-HT 933 were constructed, the α_2-adrenoceptor agonist was added to the perfusion/superfusion medium 10 min before the fifth stimulation period (S_5) and maintained throughout. Since the effect of B-HT 933 did not increase from S_5 to S_6, the S_5/S_4 ratios are indicated. Figure 3 shows that B-HT 933 concentration-dependently decreased the stimulation-evoked tritium overflow. Moreover, B-HT 933 slightly enhanced the basal tritium outflow (b_5/b_4) at 10 and 30 μmol/l (1.03 ± 0.01; $n = 8$; $P < 0.05$ and 1.08 ± 0.01; $n = 7$; $P < 0.01$, respectively) as compared to control arteries (0.98 ± 0.01; $n = 8$). The control stimulation-induced vasoconstriction (S_5/S_4; 0.99 ± 0.02; $n = 8$) was also significantly and concentration-dependently depressed by B-HT 933 10 and 30 μmol/l (0.81 ± 0.06; $n = 8$; $P < 0.05$ and 0.64 ± 0.07; $n = 7$; $P < 0.01$, respectively).

Interaction of forskolin with B-HT 933 on the electrically-evoked tritium overflow

In these experiments, forskolin was added to the perfusion/superfusion medium 10 min before S_3 and was present for the rest of the experiment. B-HT 933 was added 10 min before the fifth stimulation period (S_5) and was also maintained thereafter. Addition of forskolin 3 μmol/l 10 min before S_3 significantly increased the stimulation-evoked overflow of tritium (S_3/S_2: 1.41 ± 0.02; $n = 18$; $P < 0.01$; all appropriate experiments pooled) as compared to the control values (1.02 ± 0.02; $n = 16$; all appropriate experiments pooled). This increase remained constant during the subsequent stimulation periods, resulting in an S_5/S_4 ratio of close to unity (Fig. 4). Further addition of B-HT 933 30 μmol/l 10 min before S_5 produced a similar degree of inhibition as was seen in the control group (Fig. 4).

Interaction of 8-Br-cAMP with B-HT 933 and rauwolscine on the electrically-evoked tritium overflow

Addition of 8-Br-cAMP 100 and 300 μmol/l 10 min before S_3 resulted in a significant facilitation of the stimulation-evoked tritium overflow; this effect was stable during the following stimulation periods (see Fig. 1) resulting in an S_5/S_4 ratio of close to unity (Fig. 5). When subsequently B-HT 933 30 μmol/l was added to the medium 10 min before S_5, the relative inhibitory effect of the α_2-adrenoceptor agonist on the release of tritium was slightly but significantly decreased in comparison with the 8-Br-cAMP-free condition (Fig. 5). However, in the presence of 8-Br-cAMP 100 and 300 μmol/l the S_4 values (0.386 ± 0.037% of tissue tritium; $n = 7$; $P < 0.01$ and 0.535 ± 0.047% of tissue tritium; $n = 7$; $P < 0.01$) were significantly higher than in control experiments (0.204 ± 0.007% of tissue tritium; $n = 7$). In the presence of 8-Br-cAMP 100 μmol/l throughout the experiment, the relative enhancing effect of rauwolscine 1 μmol/l added 10 min before S_3 on the stimulation-evoked tritium overflow was significantly more pronounced (S_3/S_2: 3.20 ± 0.23; $n = 7$; $P < 0.05$) than in the absence of this compound (S_3/S_2: 2.58 ± 0.05; $n = 12$). Again in the

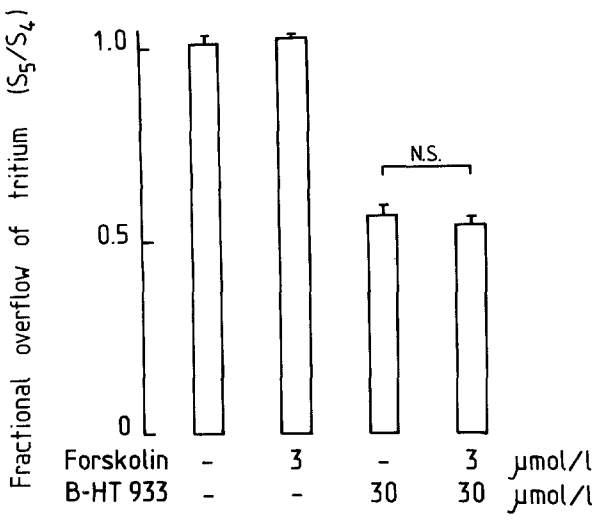

Fig. 4. Effects of forskolin and B-HT 933 applied alone or in combination on the electrically-evoked tritium overflow in rat tail arteries pre-incubated with [^{3}H]-noradrenaline. 6 periods (S_1-S_6) of field stimulation were delivered at intervals of 16 min (24 pulses at 0.4 Hz, 0.3 ms, 200 mA). Forskolin or its solvent was added 10 min before S_3. B-HT 933 or its solvent was added 10 min before S_5 in the continued presence of forskolin or its solvent. All compounds were maintained in the medium for the duration of the experiment. The tritium overflow evoked by S_4 (before the application of B-HT 933) was $0.224 \pm 0.016\%$ of tissue tritium ($n = 16$; all appropriate experiments pooled) and $0.300 \pm 0.017\%$ of tissue tritium ($n = 18$; all appropriate experiments pooled) respectively in the absence and in the presence of forskolin 3 μmol/l. The effects of the drugs are presented as the ratios of tritium overflow evoked by S_5 over that evoked by S_4. Means ± SEM from 8–10 arteries. There was no statistically significant difference (N.S.) between the indicated values

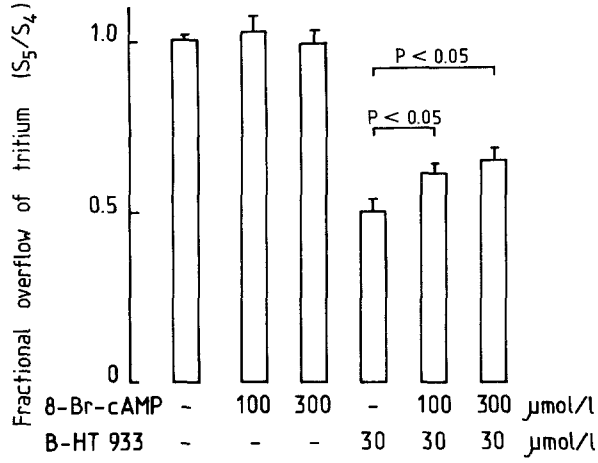

Fig. 5. Effects of 8-Br-cAMP and B-HT 933 applied alone or in combination on the electrically-evoked tritium overflow in rat tail arteries pre-incubated with [^{3}H]-noradrenaline. 6 periods (S_1-S_6) of field stimulation were delivered at intervals of 16 min (24 pulses at 0.4 Hz, 0.3 ms, 200 mA). 8-Br-cAMP, 100 or 300 μmol/l, or its solvent was added 10 min before S_3. B-HT 933 30 μmol/l, or its solvent was added 10 min before S_5 in the continued presence of 8-Br-cAMP 100 or 300 μmol/l, or its solvent. All compounds were maintained in the medium for the duration of the experiment. The tritium overflow evoked by S_4 was $0.206 \pm 0.009\%$ of tissue tritium ($n = 15$; all appropriate experiments pooled) in the absence of 8-Br-cAMP and $0.378 \pm 0.026\%$ ($n = 13$; all appropriate experiments pooled) and $0.469 \pm 0.029\%$ of tissue tritium ($n = 15$; all appropriate experiments pooled) respectively in the presence of 100 and 300 μmol/l 8-Br-cAMP. The effects of the drugs are presented as the ratios of the tritium overflow evoked by S_5 over that evoked by S_4. Means ± SEM from 7–8 arteries. The statistically significant differences between some values are indicated

presence of 8-Br-cAMP 100 μmol/l the S_2 value ($0.388 \pm 0.026\%$ of tissue tritium; $n = 7$; $P < 0.01$) was significantly higher than in control experiments ($0.235 \pm 0.013\%$ of tissue tritium; $n = 12$). Thus, the change in transmitter release produced by the first compound (in this case 8-Br-cAMP) added throughout perfusion/superfusion might be responsible for the observed diminished response to B-HT 933 or for the enhanced response to rauwolscine. In order to compensate for this change, we performed experiments, in which the stimulation conditions were altered so that in spite of the presence of 8-Br-cAMP 100 μmol/l, the tritium overflow was comparable to that obtained in the predrug situation. When the stimulation was, instead of the usual 24 pulses at 0.4 Hz and 200 mA, with only 12 pulses at 0.2 Hz and 150 mA, the tritium overflow was significantly reduced (Fig. 6). However, when during stimulation with these modified parameters 8-Br-cAMP 100 μmol/l was present, the tritium overflow was similar to that measured before application of the cyclic AMP analogue. Under these conditions, the inhibitory effect of B-HT 933 30 μmol/l was not changed by 8-Br-cAMP 100 μmol/l (Fig. 7). Similarly, the effect of B-HT 933 30 μmol/l was the same, both when 12 pulses were delivered at 0.2 Hz and 150 mA in the presence of 8-Br-cAMP 100 μmol/l (S_5/S_4: 0.47 ± 0.02; $n = 7$) and when 24 pulses were delivered at 0.4 Hz and 200 mA in the absence of 8-Br-cAMP (S_5/S_4: 0.50 ± 0.04; $n = 7$).

When the facilitatory effect of 8-Br-cAMP 100 μmol/l was compensated for by modifying the stimulation parameters (S_1-S_4: 12 pulses at 0.2 Hz, 150 mA), the stimulation-evoked tritium overflow produced by rauwolscine 1 μmol/l was significantly greater than in control (Fig. 8). However the α_2-adrenoceptor antagonist produced the same relative enhancement of the tritium overflow (S_3/S_2: 2.55 ± 0.12; $n = 6$) as it had in the absence of 8-Br-cAMP 100 μmol/l (S_3/S_2: 2.58 ± 0.05; $n = 12$) under the initial stimulation conditions. It should be noted that the overflow of tritium evoked by stimulation with 24 pulses at 200 mA, 0.4 Hz ($0.235 \pm 0.013\%$ of tissue tritium; $n = 12$) or 12 pulses at 150 mA, 0.2 Hz in the presence of 8-Br-cAMP 100 μmol/l ($0.274 \pm 0.019\%$ of tissue tritium; $n = 6$) were similar.

Discussion

In rat tail arteries preloaded with [^{3}H]-noradrenaline, the permeant analogue of cyclic AMP, 8-Br-cAMP, as well as forskolin, which is thought to act directly on the catalytic subunit of adenylate cyclase (Seamon and Daly 1983), concentration-dependently augmented the electrically-induced tritium overflow. This finding is in agreement with results obtained in other isolated peripheral tissues (Wooten et al. 1973; Cubeddu et al. 1975; Stjärne et al. 1979; Göthert and Hentrich 1984; Alberts et al. 1985;

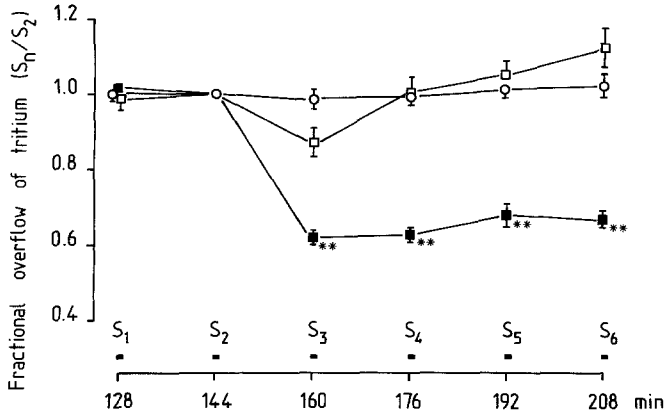

Fig. 6. Time-course of the effect of 8-Br-cAMP 100 μmol/l on the electrically-evoked tritium overflow in rat tail arteries preincubated with [^{3}H]-noradrenaline. 6 periods (S_1-S_6) of field stimulation were delivered at intervals of 16 min. In some experiments, all stimulation periods consisted of 24 pulses at 0.4 Hz, 0.3 ms, 200 mA (○: solvent). In further experiments, after S_2 the stimulation parameters were changed to 12 pulses at 0.2 Hz, 0.3 ms, 150 mA (■: solvent; □: 8-Br-cAMP 100 μmol/l). 8-Br-cAMP or its solvent was added 10 min before S_3 and maintained in the medium for the duration of the experiment. The tritium overflow evoked by S_2 (before the application of 8-Br-cAMP) was $0.232 \pm 0.008\%$ of tissue tritium ($n = 22$; all appropriate experiments pooled). The effect of the drug is presented as the ratios of tritium overflow evoked by any stimulation period (S_n) over that evoked by S_2. Means ± SEM from 7–8 arteries. Significant differences from each of the experimental groups vs the group with solvent and stimulated with 24 pulses at 0.4 Hz: ** $P < 0.01$

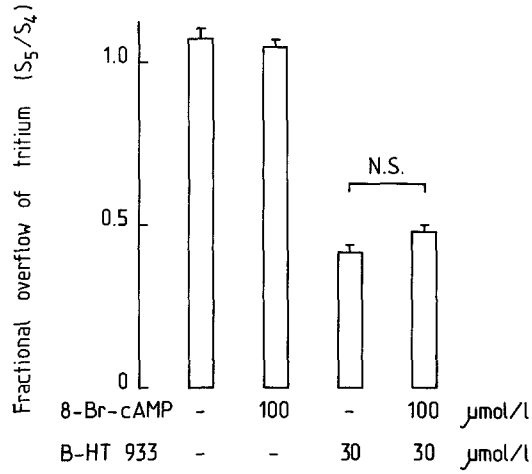

Fig. 7. Effects of 8-Br-cAMP and B-HT 933 applied alone or in combination on the electrically-evoked tritium overflow in rat tail arteries pre-incubated with [^{3}H]-noradrenaline. 6 periods (S_1-S_6) of field stimulation were delivered at intervals of 16 min. After S_2 the stimulation parameters were changed from 24 pulses at 0.4 Hz, 0.3 ms, 200 mA to 12 pulses at 0.2 Hz, 0.3 ms, 150 mA. 8-Br-cAMP or its solvent was added 10 min before S_3. B-HT 933 or its solvent was added 10 min before S_5 in the continued presence of 8-Br-cAMP or its solvent. All compounds were maintained in the medium for the duration of the experiment. The tritium overflow evoked by S_4 (before the application of B-HT 933) was $0.163 \pm 0.005\%$ of tissue tritium ($n = 15$; all appropriate experiments pooled) and $0.235 \pm 0.009\%$ of tissue tritium ($n = 14$; all appropriate experiments pooled) respectively in the absence and presence of 8-Br-cAMP 100 μmol/l. The effects of the drugs are presented as the ratios of the tritium overflow evoked by S_5 over that evoked by S_4. Means ± SEM from 7–8 arteries. There was no statistically significant difference (N.S.) between the indicated values

Hentrich et al. 1985; Johnston et al. 1987) and in brain slices (Wemer et al. 1982; Markstein et al. 1984; Schoffelmeer et al. 1985, 1986). The facilitatory effect of an increase in the intracellular level of cyclic AMP is corroborated by experiments in which phosphodiesterase inhibitors, that prevent the breakdown of endogenously synthesized cyclic nucleotides, have been shown to increase the stimulation-evoked release of noradrenaline in sympathetically innervated tissues (Wooten et al. 1973; Cubeddu et al. 1975; Stjärne et al. 1979; Wemer et al. 1982; Göthert and Hentrich 1984; Alberts et al. 1985; Hentrich et al. 1985; Markstein et al. 1984; Schoffelmeer et al. 1985, 1986; Johnston and Majewski 1986; Johnston et al. 1987). In the present study the selective cyclic AMP phosphodiesterase inhibitor, rolipram (Lugnier et al. 1986) also enhanced the [^{3}H]-noradrenaline release, although its effect was less pronounced than that of 8-Br-cAMP or forskolin. Rolipram is devoid of antagonistic properties at adenosine receptors (Schwabe et al. 1976).

Forskolin facilitated the overflow with a higher potency, but to a lower maximum value than 8-Br-cAMP. It is possible that the prejunctional capacity to synthesize cyclic AMP and, thereby, the maximum effect of forskolin, might be limited, so that the application of 8-Br-cAMP raised the intraneuronal cyclic AMP content more efficiently than forskolin. In fact, in the presence of rolipram, forskolin facilitated the release of [^{3}H]-noradrenaline to a larger extent, presumably because cyclic AMP generated by adenylate cyclase was less rapidly degraded by the phosphodiesterase. Similar findings with another cyclic AMP phosphodiesterase inhibitor (AH 21-132) plus forskolin have been observed in rat brain cortex slices (Schlicker et al. 1987) and rabbit pulmonary artery (Göthert and Hentrich 1984).

In agreement with previous reports (Göthert and Hentrich 1984; Markstein et al. 1984; Alberts et al. 1985), forskolin, rolipram and the highest concentrations of 8-Br-cAMP enhanced the basal tritium outflow only slightly. The enhancement by these compounds of the nerve stimulation-evoked release of [^{3}H]-noradrenaline was much larger, suggesting that cyclic AMP does not initiate transmitter release by itself, but that it is one of the factors involved in the modulation of the stimulation-evoked release.

The adenylate cyclase activator forskolin has been shown to produce a concentration-dependent reversible relaxation of isolated smooth muscle preparations including blood vessels (Muller and Baer 1983; Göthert and Hentrich 1984). Our data confirm these observations and extend them to rolipram. However, since 8-Br-cAMP elicited a slight concentration-dependent increase in the stimulation-evoked vasoconstriction, this result seems to contrast with the relaxant effect of forskolin and rolipram. In this respect it should be considered that the terminal axons of postganglionic sympathetic nerves innervating blood vessels are embedded in a tissue which consists mainly of smooth muscle cells. The effect of drugs on the vasoconstriction elicited by nerve stimulation is the net result of their pre- and postjunctional actions. It

has previously been suggested (Göthert and Hentrich 1984) that the differences observed between the effects on the electrically-induced vasoconstriction of 8-Br-cAMP on the one hand, and forskolin or rolipram on the other, may reflect differences in the sensitivity of the pre- and postjunctional target systems and/or differences in the ability of the drugs to diffuse into the respective cell compartment. It is also possible that the contractile effects of the two co-transmitters, noradrenaline and ATP (Sneddon and Burnstock 1984; Bao et al. 1989) are differentially influenced by changes in the intracellular levels of cAMP. An alternative explanation is that forskolin dilates blood vessels by opening potassium channels in the smooth muscle cell membrane (Encina et al. 1988; Hoshi et al. 1988). Such an effect was shown to be independent of any effect on the adenylate cyclase, since 1,9-dideoxy-forskolin (Seamon and Daly 1986) increases a resting potassium conductance without enhancing the concentration of cyclic AMP (Hoshi et al. 1988; Ertl and Nawrath 1989). The inability of 1,9-dideoxy-forskolin to decrease the vasoconstriction of rat tail arteries strongly argues against a non-cyclic AMP-mediated relaxant effect of forskolin.

Nerve terminals in peripheral organs and in the central nervous system are endowed with various receptors. Notable among these are the prejunctional α_2-adrenoceptors involved in the autoinhibition of noradrenaline release during nerve stimulation (Langer 1981; Starke 1981, 1987). The question to be answered was whether adenylate cyclase is negatively coupled to the prejunctional α_2-adrenoceptors (see Mulder and Schoffelmeer 1985; Illes 1986). In our study the selective α_2-adrenoceptor agonist B-HT 933 (Andén et al. 1982; Wilffert et al. 1984) concentration-dependently depressed the stimulation-induced release of [^{3}H]-noradrenaline, and the selective α_2-adrenoceptor antagonist rauwolscine (Weitzell et al. 1979) at 1 μmol/l increased the release evoked by nerve stimulation, when applied alone. In addition, rauwolscine 1 μmol/l, abolished the inhibitory effect of B-HT 933 10 μmol/l (Weber 1989; Weber, Allgaier, Hertting and Illes, unpublished observation). Thus, the facilitation by rauwolscine may be due to the interruption of an α_2-adrenoceptor-mediated feed-back control imposed upon transmitter release (see Langer 1981; Starke 1981, 1987).

In the central nervous system, prejunctional α_2-adrenoceptors situated at the terminals of both noradrenergic (Schoffelmeer and Mulder 1983; Schoffelmeer et al. 1985) and serotoninergic (Schlicker et al. 1987) neurones are believed to be linked to adenylate cyclase. In apparent support of this concept, we found in a sympathetically innervated smooth muscle preparation, the rat tail artery, that 8-Br-cAMP slightly but significantly attenuated the inhibitory effect of the α_2-agonist B-HT 933 on [^{3}H]-noradrenaline release. If the effect were due to a reversal of the α_2-adrenoceptor mediated inhibition of adenylate cyclase, the relative facilitatory effect of rauwolscine should have been attenuated in the presence of 8-Br-cAMP due to less feedback inhibition through prejunctional α_2-adrenoceptors. This was not the case, on the contrary the relative facilitation by rauwolscine was increased by the addition of 8-Br-cAMP. Previous results obtained in a wide variety of peripheral organs (Cubeddu et al. 1975; Alberts et al. 1985; Johnston et al. 1987) have led to the concept that, at least in postganglionic sympathetic neurones, α_2-adrenoceptor-mediated inhibition of noradrenaline release is not due to inhibition of adenylate cyclase. Thus, we assumed that the apparent decrease in the effect of B-HT 933 and the relative higher enhancement of the facilitatory effect of rauwolscine observed in the presence of 8-Br-cAMP could be due to the increase in transmitter release produced by the cyclic nucleotide. In fact, it has been shown that the inhibitory effect of B-HT 933 on the release of noradrenaline from superfused brain slices is diminished at high biophase levels of the transmitter (Cichini and Singer 1987). We therefore thought it important to ascertain that changes in the prejunctional effect of B-HT 933 are not a consequence of the enhanced release of [^{3}H]-noradrenaline by 8-Br-cAMP. This important prerequisite has been well documented (Starke 1987; Limberger et al. 1988). We, therefore, designed experiments in which, after the addition of 8-Br-cAMP 100 μmol/l, the stimulation parameters were adjusted so that the release of [^{3}H]-noradrenaline was the same as before the application of the cyclic nucleotide analogue. Under these conditions, the inhibitory effect of the α_2-adrenoceptor agonist B-

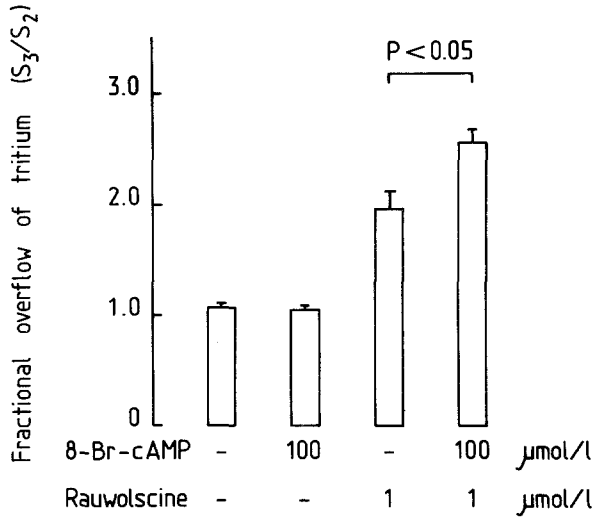

Fig. 8. Effects of 8-Br-cAMP and rauwolscine applied alone or in combination on the electrically-evoked tritium overflow in rat tail arteries pre-incubated with [^{3}H]-noradrenaline. 4 periods (S_1-S_4) of field stimulation were delivered at intervals of 16 min (12 pulses at 0.2 Hz, 0.3 ms, 150 mA). 8-Br-cAMP or its solvent was added 10 min before S_1. Rauwolscine or its solvent was added 10 min before S_3 in the continued presence of 8-Br-cAMP or its solvent. All compounds were maintained in the medium for the duration of the experiment. The tritium overflow evoked by S_2 (before the application of rauwolscine) was $0.172 \pm 0.008\%$ of tissue tritium ($n = 14$; all appropriate experiments pooled) and $0.253 \pm 0.013\%$ of tissue tritium ($n = 13$; all appropriate experiments pooled) respectively in the absence and presence of 8-Br-cAMP 100 μmol/l. The effects of the drugs are presented as the ratios of the tritium overflow evoked by S_3 over that evoked by S_2. Means ± SEM from 6–7 arteries. The statistically significant difference between some values is indicated

HT 933 and the facilitatory effect of the α_2-adrenoceptor antagonist rauwolscine were not altered by 8-Br-cAMP as compared to control experiments under initial stimulation parameters. It is, however, difficult to explain why, in contrast to 8-Br-cAMP, forskolin did not antagonize B-HT 933 even when the stimulation parameters were kept constant, although forskolin increased the biophase concentration of noradrenaline only to a slightly lower extent than 8-Br-cAMP did. It is possible that there is a threshold concentration of endogenous noradrenaline in the neighbourhood of the prejunctional receptors, above which the effects of exogenous α_2-agonists are decreased. Nevertheless, the present data obtained by forskolin fail to support the existence of a negative link between prejunctional α_2-adrenoceptors and adenylate cyclase in perivascular nerve terminals.

Altogether, the present results obtained in a blood vessel, the rat tail artery, are in good agreement with those obtained in other non-vascular peripheral tissues and are consistent with the view that the α_2-adrenoceptor modulation of stimulated noradrenaline release from peripheral sympathetic nerve endings is not produced by inhibition of adenylate cyclase.

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References

Alberts P, Ögren VR, Sellström AI (1985) Role of adenosine 3′,5′-cyclic monophosphate in adrenoceptor-mediated control of ^{3}H-noradrenaline secretion in guinea-pig ileum myenteric nerve terminals. Naunyn-Schmiedeberg's Arch Pharmacol 330:114–120

Andén NE, Golembiowska-Nikitin K, Thornström U (1982) Selective stimulation of dopamine and noradrenaline autoreceptors by B-HT 920 and B-HT 933, respectively. Naunyn-Schmiedeberg's Arch Pharmacol 321:100–104

Bao JX, Eriksson IE, Stjärne L (1989) Neurogenic contractions in the rat tail artery of normotensive rats are mediated by noradrenaline and ATP via post-junctional α_1- and α_2-adrenoceptors and P_{2x}-purinoceptors. Acta Physiol Scand 136:139–140

Blaustein MP (1979) The role of calcium in catecholamine release from adrenergic nerve terminals. In: Paton DM (ed) The release of catecholamines from adrenergic neurons. Pergamon, Oxford, pp 39–58

Brown AM, Birnbaumer L (1988) Direct G protein gating of ion channels. Am J Physiol 245:H401–H410

Bucher B, Bettermann R, Illes P (1987) Plasma concentration and vascular effect of β-endorphin in spontaneously hypertensive and Wistar Kyoto rats. Naunyn-Schmiedeberg's Arch Pharmacol 335:428–432

Cichini G, Singer EA (1987) B-HT 920, B-HT 933, and B-HT 958: presynaptic effects on electrically evoked ^{3}H-noradrenaline release from slices of rat brain cortex and hypothalamus. Naunyn-Schmiedeberg's Arch Pharmacol 335:613–617

Cubeddu L, Barnes E, Weiner N (1975) Release of norepinephrine and dopamine β-hydroxylase by nerve stimulation. IV. An evaluation of a role for cyclic adenosine monophosphate. J Pharmacol Exp Ther 193:105–127

Encina JL, Hartung F, Tripathi O, Nawrath H (1988) Analysis of the hyperpolarizing effects of forskolin in guinea-pig atrial heart muscle. Naunyn-Schmiedeberg's Arch Pharmacol 337:435–439

Ertl R, Nawrath H (1989) Cyclic AMP-independent membrane effects of forskolin in atrial and ventricular heart and aortic smooth muscle preparations from guinea pigs. Naunyn-Schmiedeberg's Arch Pharmacol Suppl 339:R52

Exton JH (1982) Molecular mechanisms involved in α-adrenergic responses. Trends Pharmacol Sci 3:111–115

Göthert M, Hentrich F (1984) Role of cAMP for regulation of impulse-evoked noradrenaline release from rabbit pulmonary artery and its possible relationship to presynaptic ACTH receptors. Naunyn-Schmiedeberg's Arch Pharmacol 328:127–134

Hentrich F, Göthert M, Greschuchna D (1985) Involvement of cAMP in modulation of noradrenaline release in the human pulmonary artery. Naunyn-Schmiedeberg's Arch Pharmacol 330:245–247

Hoshi T, Garber SS, Aldrich RW (1988) Effect of forskolin on voltage-gated K^+ channels is independent of adenylate cyclase activation. Science 240:1652–1655

Illes P (1986) Mechanisms of receptor-mediated modulation of transmitter release in noradrenergic, cholinergic and sensory neurones. Neuroscience 17:909–928

Illes P, Bettermann R, Brod I, Bucher B (1987) β-Endorphin-sensitive opioid receptors in the rat tail artery. Naunyn-Schmiedeberg's Arch Pharmacol 335:420–427

Jakobs KH, Aktories K, Schultz G (1981) Inhibition of adenylate cyclase by hormones and neurotransmitters. Adv Cyclic Nucleotide Res 14:173–186

Johnston H, Majewski H (1986) Prejunctional β-adrenoceptors in rabbit pulmonary artery and mouse atria: effect of α-adrenoceptor blockade and phosphodiesterase inhibition. Br J Pharmacol 87:553–562

Johnston H, Majewski H, Musgrave IF (1987) Involvement of nucleotides in prejunctional modulation of noradrenaline release in mouse atria. Br J Pharmacol 91:773–781

Langer SZ (1981) Presynaptic regulation of the release of catecholamines. Pharmacol Rev 32:337–362

Limberger N, Späth L, Starke K (1988) Presynaptic α_2-adrenoceptor, opioid κ-receptor and adenosine A_1-receptor interactions on noradrenaline release in rabbit brain cortex. Naunyn-Schmiedeberg's Arch Pharmacol 338:53–61

Lugnier C, Schoeffter P, Le Bec A, Strouthou E, Stoclet JC (1986) Selective inhibition of cyclic nucleotide phosphodiesterases of human, bovine and rat aorta. Biochem Pharmacol 35:1743–1751

Markstein R, Digges K, Marshall NR, Starke K (1984) Forskolin and the release of noradrenaline in cerebrocortical slices. Naunyn-Schmiedeberg's Arch Pharmacol 325:17–24

Mattera R, Graziano MP, Yatani A, Zhou Z, Graf R, Codina J, Birnbaumer L, Gilman AG, Brown AM (1989) Splice variants of the α subunit of the G protein G_s activate both adenylyl cyclase and calcium channels. Science 243:804–807

Mulder AH, Schoffelmeer ANM (1985) Catecholamine and opioid receptors, presynaptic inhibition of CNS neurotransmitter release, and adenylate cyclase. Adv Cycl Nucleotide Protein Phosphoryl Res 19:273–286

Muller MJ, Baer HP (1983) Relaxant effects of forskolin in smooth muscle. Role of cyclic AMP. Naunyn-Schmiedeberg's Arch Pharmacol 322:78–82

Neer EJ, Clapham DE (1988) Roles of G protein subunits in transmembrane signalling. Nature 333:129–134

Rodbell M (1980) The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 284:17–22

Schlicker E, Fink K, Classen K, Göthert M (1987) Facilitation of serotonin (5-HT) release in the rat brain cortex by cAMP and probable inhibition of adenylate cyclase in 5-HT nerve terminals by presynaptic α_2-adrenoceptors. Naunyn-Schmiedeberg's Arch Pharmacol 336:251–256

Schoffelmeer ANM, Mulder AH (1983) ^{3}H-Noradrenaline release from rat neocortical slices in the absence of extracellular Ca^{2+} and its presynaptic alpha$_2$-adrenergic modulation. A study on the possible role of cyclic AMP. Naunyn-Schmiedeberg's Arch Pharmacol 323:188–192

Schoffelmeer ANM, Hogenboom F, Mulder AH (1985) Evidence for a presynaptic adenylate cyclase system facilitating [^{3}H]-norepinephrine release from rat brain neocortex slices and synaptosomes. J Neurosci 5:2685–2689

Schoffelmeer ANM, Wierenga EA, Mulder AH (1986) Role of adenylate cyclase in presynaptic α_2-adrenoceptor- and μ-opioid receptor-mediated inhibition of [^{3}H]noradrenaline release from rat brain cortex slices. J Neurochem 46:1711–1717

Schwabe U, Miyake M, Ohga Y, Daly JW (1976) 4-(3-Cyclopentyloxy-4-methoxy-phenyl)-2-pyrrolidone (ZK 62711): A potent inhibitor of cyclic AMP-phosphodiesterases in homogenates and tissue slices from rat brain. Mol Pharmacol 12:900–910

Seamon KB, Daly JW (1983) Forskolin, cyclic AMP and cellular physiology. Trends Pharmacol Sci 4:120–123

Seamon KB, Daly JW (1986) Forskolin: Its biological and chemical properties. Adv Cyclic Nucleotide Protein Phosphorylation Res 20:1–150

Sneddon P, Burnstock G (1984) ATP as a co-transmitter in rat tail artery. Eur J Pharmacol 106:149–152

Starke K (1981) Presynaptic receptors. Ann Rev Pharmacol Toxicol 21:7–30

Starke K (1987) Presynaptic α-autoreceptors. Rev Physiol Pharmacol 107:73–146

Stjärne L, Bartfai T, Alberts P (1979) The influence of 8-Br 3′,5′-cyclic nucleotide analogs and of inhibitors of 3′,5′-cyclic nucleotide phosphodiesterase, on noradrenaline secretion and neuromuscular transmission in guinea-pig vas deferens. Naunyn-Schmiedeberg's Arch Pharmacol 308:99–105

Weber HD (1989) Presynaptic α_2-adrenoceptors at the terminals of perivascular sympathetic nerves are coupled to an N-ethylmaleimide-sensitive G-protein. Naunyn-Schmiedeberg's Arch Pharmacol Suppl 339:R 35

Weitzell R, Tanaka T, Starke K (1979) Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. Naunyn-Schmiedeberg's Arch Pharmacol 308:127–136

Wemer J, Schoffelmeer ANM, Mulder AH (1982) Effects of cyclic AMP analogues and phosphodiesterase inhibitors on K^+-induced [^{3}H]noradrenaline release from rat brain slices and on its presynaptic α-adrenergic modulation. J Neurochem 39:349–356

Wilffert B, Smit G, de Jonge A, Thoolen MJMC, Timmermans PBMWM, van Zwieten PA (1984) Inhibitory dopamine receptors on sympathetic neurons innervating the cardiovascular system of the pithed rat. Characterization and role in relation to presynaptic α_2-adrenoceptors. Naunyn-Schmiedeberg's Arch Pharmacol 326:91–98

Wooten GF, Thoa NB, Kopin IJ, Axelrod J (1973) Enhanced release of dopamine β-hydroxylase and norepinephrine from sympathetic nerves by dibutyryl cyclic adenosine 3′,5′-monophosphate and theophylline. Mol Pharmacol 9:178–183