Naunyn-Schmiedeberg's Arch Pharmacol (1984) 325:17–24

Naunyn-Schmiedeberg's
Archives of
Pharmacology

Forskolin and the release of noradrenaline in cerebrocortical slices

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Summary. The role of adenosine 3′,5′-cyclic monophosphate (cAMP) in the release of noradrenaline from central neurones has been investigated by examining the effects of forskolin, 3-isobutyl-l-methylxanthine (IBMX), cis-6-(p-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo[c][1,6]-naphthyridine bis(hydrogenmaleinate) (AH21-132; a new phosphodiesterase inhibitor) and N^6,$O^{2'}$-dibutyryl-adenosine 3′,5′-cyclic monophosphate (dibutyryl-cAMP) on the outflow of tritiated compounds from rat and rabbit cerebral cortex slices preincubated with [^{3}H]-noradrenaline. Forskolin, IBMX, AH21-132 and dibutyryl-cAMP produced a concentration-dependent increase in both basal and electrically-evoked efflux of tritium from rat and rabbit cortex slices. The increase in basal tritium efflux from rabbit cortex slices elicited by forskolin and IBMX could be attributed mainly to an increase in [^{3}H]-DOPEG although a small increase in [^{3}H]-noradrenaline was also observed. Forskolin and (when combined with noradrenaline) IBMX and AH21-132 increased the cAMP content of rat cortex slices at similar or somewhat higher concentrations that they increased tritium efflux. Neither forskolin nor IBMX or AH21-132 had any effect on the cocaine-sensitive uptake of [^{3}H]-noradrenaline into synaptosomes prepared from rat or rabbit cortex. The effects of forskolin, IBMX and dibutyryl-cAMP on electrically-evoked overflow of tritium from rat and rabbit cortex slices were reduced when cocaine (10 μM) was present in the superfusion medium, although forskolin produced a similar increase in cAMP in the absence or presence of cocaine. It is suggested that cAMP may facilitate the normal process of noradrenaline release by nerve stimulation.

Key words: Cyclic AMP – Noradrenaline release – Cerebral noradrenaline neurones – Forskolin – Phosphodiesterase inhibitors

Introduction

The role played by adenosine 3′,5′-cyclic monophosphate (cAMP) in neuronal catecholamine release is still uncertain. In the case of the nigro-striatal dopamine neurones, cAMP does not seem to affect the release of the transmitter (Patrick and Barchas 1976; Eitan and Hershkowitz 1977; Cubeddu et al. 1979; Reisine et al. 1982; see, however, Westfall et al. 1976) but increases its biosynthesis (see Reisine et al. 1982). In contrast, in the case of postganglionic sympathetic neurons, cAMP, although not a necessary prerequisite for the action potential-evoked release of noradrenaline, facilitates it, as indicated by the increase in release caused by cAMP analogues and phosphodiesterase (PDE) inhibitors (see, e.g., Cubeddu et al. 1975; Pelayo et al. 1978; Stjärne et al. 1979; for review Starke 1977; Stjärne 1979). Finally, and again in surprising contrast, in the case of cerebrocortical noradrenaline neurones, potassium-evoked transmitter release was enhanced by the cAMP analogues N^6,$O^{2'}$-dibutyryl-adenosine 3′,5′-cyclic monophosphate (dibutyryl-cAMP) and 8-bromoadenosine 3′,5′-cyclic monophosphate (Wemer et al. 1982; Schoffelmeer and Mulder 1983; see, however, Eitan and Hershkowitz 1977), but was reduced or tended to be reduced by PDE inhibitors including 3-isobutyl-l-methylxanthine (IBMX; Dismukes and Mulder 1976; Eitan and Hershkowitz 1977; Ebstein et al. 1982; Wemer et al. 1982; Schoffelmeer and Mulder 1983).

Recently with forskolin a new tool has become available, suitable to study the involvement of cAMP in physiological events. Forskolin is a naturally occurring diterpene which activates adenylate cyclase in intact tissue as well as in broken cell preparations (Metzger and Lindner 1981). The activation probably occurs via an interaction with the catalytic subunit of adenylate cyclase (for review see Seamon and Daly 1983).

The present experiments were designed to examine the effects of forskolin and other drugs related to cAMP on the release of noradrenaline in slices of the cerebral cortex from rats and rabbits. In contrast to previous investigations (Dismukes and Mulder 1976; Eitan and Hershkowitz 1977; Ebstein et al. 1982; Wemer et al. 1982), release was elicited by electrical stimulation. Forskolin has already been shown to stimulate renin release from the rat kidney (Schwertschlag and Hackenthal 1982), to increase electrically-evoked vasopressin release from rat pituitary (Racké et al. 1982), and to stimulate the conversion of tyrosine to dopamine in neural tissue (Katz et al. 1983).

Methods

General. Male rats (Sandoz OFA strain, weighing approximately 150 g) and rabbits of either sex (weighing approximately 2 kg) were used. The animals were killed by decapitation and the brains rapidly removed and dissected over a chilled plate.

The medium used for incubation and superfusion in rat experiments consisted of (mM): NaCl 118, KCl 5, $CaCl_2$ 1.2,

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$MgCl_2$ 2, $NaHCO_3$ 25, KH_2PO_4 2, glucose 11, Na_2EDTA 0.02, with appropriate additions as indicated. The medium used in rabbit experiments consisted of (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, Na_2EDTA 0.02, ascorbic acid 0.57, with appropriate additions as indicated. Media were saturated with 5% CO_2 in O_2.

Tritium was determined by liquid scintillation counting. Means ± SE of *n* experiments are given throughout. Significance of differences was calculated with the two-tailed *t*-test.

Tritium outflow from rat cerebral cortex slices preincubated with [^{3}H]-noradrenaline. Slices of approximately 0.34 × 0.34 × 1 mm were prepared from rat occipital cortex, using a McIlwain tissue chopper. The slices obtained from 3 rats (approximately 200 mg) were incubated for 30 min at room temperature in 6 ml medium containing 0.1 µM (–)-[^{3}H]-noradrenaline (specific activity 47 Ci/mmol) and 0.5 mM ascorbic acid. Subsequently, the slices were washed and resuspended in 2 ml medium. Portions of 200 µl of this suspension (approximately 20 mg wet weight) were distributed into each of 10 glass superfusion chambers, where the slices were held by a polypropylene mesh between gold electrodes 20 mm apart. The slices were superfused with medium at a rate of 1.2 ml/min at 30° C. Collection of 5-min fractions (6 ml) of the superfusate began after 60 min of superfusion. Two 2-min periods of electrical stimulation (frequency 2 Hz, rectangular pulses of 2 ms width, 12 mA current strength) were applied after 75 (S_1) and 150 (S_2) min of superfusion. Test substances were usually added 30 min before S_2 and were present in the medium between 120 and 170 min of superfusion. At the end of the experiment, the slices were solubilized in concentrated formic acid, and total tritium was measured in superfusates and solubilized slices. Tritium outflow was expressed as fractional rate per min, i.e. (nCi tritium outflow per 5 min)/5·(nCi tritium in the slice at the onset of the 5-min collection period). Stimulation-evoked tritium overflow was calculated by subtraction of the extrapolated basal outflow from the total outflow during the 2 min of stimulation and the following 13 min; the stimulation-evoked overflow was then expressed as percentage of the tritium content of the tissue at the onset of stimulation. Drug effects on the stimulation-evoked overflow were expressed as the ratio of the overflow evoked by S_2 to the overflow by S_1, i.e. S_2/S_1. Drug effects on basal tritium outflow were expressed as the ratio of the outflow 25 – 30 min after drug addition (b_2, immediately before S_2) to the outflow immediately before drug addition (b_1), i.e. b_2/b_1.

Tritium outflow from rabbit cerebral cortex slices preincubated with [^{3}H]-noradrenaline. Round slices of approximately 5 mm diameter and 0.4 mm thick (weighing about 8 mg) were prepared with a razor blade and punch from the occipital and parietal cortices, after the superficial layer (0.3 mm) had been removed. Six slices from one animal were incubated for 30 min at 37° C in 2 ml medium, containing 0.1 µM (–)-[^{3}H]-noradrenaline (specific activity 10 Ci/mmol). Subsequently, the slices were washed, and one slice was transferred into each of 6 glass superfusion chambers where it was held by a polypropylene mesh between platinum electrodes 20 mm apart. The slices were superfused with medium at a rate of 1 ml/min at 37°C. Collection of 5-min fractions (5 ml) of the superfusate began after 50 min of superfusion. Two 2-min periods of electrical stimulation (frequency 3 Hz, rectangular pulses of 2 ms with, 24 mA current strength) were applied after 60 (S_1) and 105 (S_2) min of superfusion. Test substances were usually added 25 min before S_2 and were present until the end of the experiment. At the end of the experiment, the slices were solubilized in Soluene-100 (Packard Instruments, Frankfurt am Main, FRG). Tritium outflow, stimulation-evoked tritium overflow and drug effects on evoked overflow were calculated as for rat cortical slice experiments. Drug effects on basal tritium outflow were expressed as the ratio of the outflow 20 – 25 min after drug addition (b_2, immediately before S_2) to the outflow immediately before drug addition (b_1), i.e. b_2/b_1.

In some experiments, [^{3}H]-noradrenaline and [^{3}H]-3,4-dihydroxyphenylglycol (DOPEG) were separated from other [^{3}H]-compounds. Slices were preincubated and superfused as above, but the specific activity of the [^{3}H]-noradrenaline was 43.9 Ci/mmol, one and a half slices were placed in each chamber, and electrical stimulation was omitted. The superfusates from two chambers were pooled. Drugs were added after 80 min of superfusion. [^{3}H]-noradrenaline and [^{3}H]-DOPEG were determined in addition to total tritium as described by Graefe et al. (1973). The outflow of [^{3}H]-compounds was expressed as fmol/5 min.

[^{3}H]-noradrenaline uptake. Rat or rabbit cerebral cortex was homogenized in 25 volumes (w/v) of ice-cold 0.32 M sucrose solution in a Potter-Elvehyem homogenizer with a teflon pestle by 10 strokes. The homogenate was centrifuged at 1,000 *g* for 10 min at 4° C. The pellet was discarded, the supernatant recentrifuged at 10,000 *g* for 20 min and the sediment gently resuspended in half of the original volume of the homogenate using medium containing 40 µM pargyline and 500 µM ascorbic acid. 400 µl of the crude synaptosomal suspension was added to test-tubes containing the test substances dissolved in 600 µl of medium, and the mixture was preincubated for 10 min at 30° C. 100 µl of (–)-[^{3}H]-noradrenaline solution (specific activity 43.9 Ci/mmol, final concentration about 3 nM) was then added and the reaction terminated 15 min later by filtration through glass fibre filters (Gelman Type A/E). The filters were washed twice with cold 0.9% NaCl solution and counted for radioactivity. Blank values were determined by incubating samples at 0° C.

cAMP content of rat cerebral cortex slices. Slices of approximately 0.34 × 0.34 × 1 mm were prepared from the occipital cortex. The slices obtained from 4 rats (approximately 600 mg) were suspended in 25 ml medium and gently stirred for 45 min at 30° C. Subsequently, the slices were suspended in 50 ml fresh medium and portions of 2 ml (approximately 20 mg wet weight) distributed into 5-ml test tubes. In some experiments, cocaine, IBMX or AH21-132 was then added. After further incubation for 15 min, 100 µl of noradrenaline or forskolin solution was added and the reaction terminated 8 min later by centrifugation of the test tubes at 200 *g* for 20 s. The supernatant was discarded and the sediment homogenized in cold 0.4 M $HClO_4$. After neutralization with 0.4 M $KHCO_3$ solution, cAMP in the extract was measured with a double antibody radioimmunoassay (Steiner et al. 1969; Frandsen and Krishna 1976). The cAMP content was referred to the protein content determined in each test tube by the biuret method (Beisenherz et al. 1953).

Drugs. (–)-[7,8-^{3}H]-noradrenaline (rat) and (–)-[ring-2,5,6-^{3}H]-noradrenaline (rabbit) from NEN (Dreieich, FRG);

Table 1. Effects of forskolin, IBMX and AH21-132 on basal and electrically-evoked tritium efflux from rat and rabbit cerebral cortex slices. Slices were stimulated twice (S_1, S_2). Forskolin, IBMX or AH21-132 was added 30 (rat) or 25 min (rabbit) before S_2. Values are the ratios of the basal outflows immediately before S_2 and immediately before drug addition (b_2/b_1) and of the overflows evoked by S_2 and S_1 (S_2/S_1). Means ± SE of n experiments

Drug added before S_2 (μM)	Rat			Rabbit		
	b_2/b_1	S_2/S_1	n	b_2/b_1	S_2/S_1	n
Forskolin						
0	0.94 ± 0.01	0.97 ± 0.01	10	0.82 ± 0.01	0.96 ± 0.01	14
0.01	0.91 ± 0.02	0.97 ± 0.04	4	0.88 ± 0.03*	0.97 ± 0.01	6
0.1	0.99 ± 0.01*	1.03 ± 0.02*	4	0.83 ± 0.02	1.03 ± 0.01**	6
1	1.14 ± 0.03**	1.30 ± 0.02**	10	0.90 ± 0.03*	1.14 ± 0.02**	8
10	1.25 ± 0.03**	1.46 ± 0.06**	6	1.00 ± 0.03**	1.54 ± 0.12**	8
IBMX						
0	0.98 ± 0.01	0.96 ± 0.02	14	0.85 ± 0.01	0.93 ± 0.02	8
0.1	1.02 ± 0.06	1.05 ± 0.05	6			
1	1.00 ± 0.06	1.10 ± 0.01**	10	0.85 ± 0.01	1.07 ± 0.03*	4
10	0.99 ± 0.13	1.21 ± 0.04**	8	0.85 ± 0.02	1.22 ± 0.02**	4
100	1.11 ± 0.04**	1.26 ± 0.03**	8	1.02 ± 0.06*	1.62 ± 0.09**	4
1,000	1.67 ± 0.05**	1.52 ± 0.06**	4	1.40 ± 0.02**	2.36 ± 0.06**	4
AH21-132						
0	0.94 ± 0.01	0.94 ± 0.01	4	0.79 ± 0.02	1.01 ± 0.02	10
1	0.89 ± 0.08	1.06 ± 0.05	4	0.82 ± 0.01	1.02 ± 0.03	8
10	1.10 ± 0.04*	1.24 ± 0.07*	4	0.91 ± 0.02*	1.41 ± 0.04**	6
100	2.13 ± 0.13**	2.22 ± 0.09**	4	1.81 ± 0.06**	3.09 ± 0.16**	6

Significant differences from control: $*P < 0.05$; $**P < 0.001$

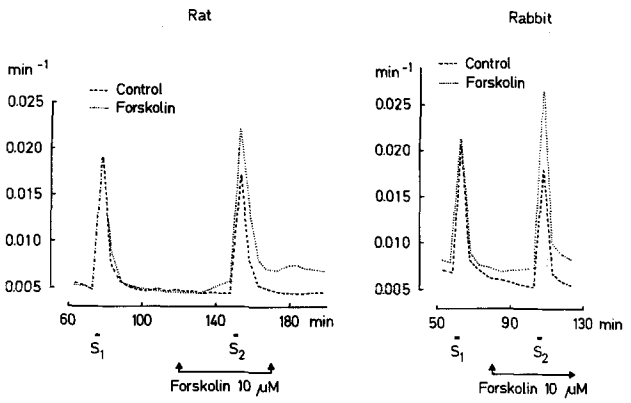

Fig. 1. Outflow of tritium from rat and rabbit cerebral cortex slices preincubated with [^{3}H]-noradrenaline, and the effect of forskolin. Slices were stimulated twice for 2 min each (S_1, S_2; rats 2 Hz, rabbits 3 Hz). In the experiments represented by *dotted lines*, forskolin (10 μM) was added as indicated. *Abscissa*, min of superfusion; *ordinate*, fractional rate of tritium outflow (min^{-1}). Means of 6–14 experiments. Standard errors were maximally 13 % of corresponding means

forskolin from Calbiochem-Behring (La Jolla, CA, USA) or Professor Lindner, Hoechst (Frankfurt am Main, FRG); 3-isobutyl-1-methylxanthine (IBMX) from Janssen (Beerse, Belgium) or Sigma (München, FRG); cis-6-(p-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo[c] [1,6]-naphthyridine bis(hydrogenmaleinate) (AH21-132) from Sandoz (Basel, Switzerland); N^6,$O^{2'}$-dibutyryladenosine 3′,5′-cyclic monophosphate (dibutyryl-cAMP) from Boehringer Mannheim (FRG); clonidine hydrochloride from Boehringer Ingelheim (FRG); (–)-noradrenaline bitartrate from Hoechst (Frankfurt am Main, FRG); pargyline hydrochloride from Abbott (Chicago, IL, USA); cocaine hydrochloride from Lehner (Muttenz, Switzerland) or Sigma (München, FRG). Drugs were dissolved in medium except forskolin which was initially dissolved in ethanol and then diluted in medium. Ethanol was also added to control medium, in the same final concentration.

Results

Tritium outflow after preincubation with [^{3}H]-noradrenaline

Electrical stimulation of rat or rabbit cortical slices previously labelled with [^{3}H]-noradrenaline produced a marked increase in tritium efflux (Fig. 1). The overflow elicited by the first stimulation period (S_1) amounted to 1.70 ± 0.05 % of tissue tritium in rat cortex slices ($n = 119$), and 1.63 ± 0.05 % in rabbit cortex slices ($n = 96$).

Forskolin added before the second stimulation period (S_2) enhanced both basal and, at similar concentrations, stimulation-evoked tritium efflux (Fig. 1, Table 1). At 10 μM, the highest concentration tested, the increase in electrically-evoked overflow amounted to 51 % of control values in the rat and 60 % in the rabbit. The PDE inhibitors IBMX and AH21-132 also increased both the basal and, at similar or already at lower concentrations, the evoked overflow (Table 1). At 1 mM, IBMX increased the electrically-evoked overflow by 58 % in the rat and by 154 % in the rabbit. At 100 μM, AH21-132 increased the evoked overflow by 136 % in the rat and by 206 % in the rabbit.

The interaction of forskolin with IBMX was then studied. In rat cortex slices, when a combination of forskolin (1 μM) and IBMX (1 μM) was added 30 min before S_2, the ratio S_2/S_1 was 1.52 ± 0.05 ($n = 6$, $P < 0.001$ versus controls without

either drug). Comparison with Table 1 shows that the effects of the two drugs were additive or slightly more than additive. In rabbit cortex slices, forskolin (1 μM) was added throughout superfusion, and various concentrations of IBMX were added before S_2 (Table 2). Note that in the presence of forskolin, the response to S_1 was augmented to 2.33 ± 0.13 % of tissue tritium ($n = 24$, $P < 0.001$). Comparison of Tables 2 and 1 shows that IBMX tended to increase the S_2/S_1 ratios more in the presence of forskolin than in its absence, indicating again that the effects of the two compounds were at least additive.

Dibutyryl-cAMP slightly but significantly increased the evoked overflow of tritium from rat cortical slices at 1 mM, the highest concentration tested. The small increase in basal efflux was not statistically significant (Table 3).

The effects of forskolin, IBMX, AH21-132 and dibutyryl-cAMP were also studied in slices superfused throughout with cocaine (10 μM). Cocaine did not change the basal outflow of tritium, but increased the evoked overflow (S_1) to 3.14 ± 0.05 % of tissue tritium in the rat ($n = 80$, $P < 0.001$) and 3.14 ± 0.05 % in the rabbit ($n = 90$, $P < 0.001$). Comparison of Tables 4 and 1 shows that cocaine did not consistently change the effects of forskolin, IBMX and AH21-132 on basal efflux of tritium. However, except for AH21-132 and rabbit cortical slices, all effects on the evoked overflow were reduced. For instance, forskolin (10 μM) increased evoked tritium overflow from rat and rabbit cortex slices by 51 and 60 %, respectively, in the absence of cocaine, but by only 11 and 20 % in the presence of cocaine. IBMX (1 mM) increased the evoked tritium overflow in rat and rabbit by 58 and 154 %, respectively, in the absence of cocaine, but by only 21 and 100 % in its presence. Cocaine abolished the small increase in evoked overflow caused by dibutyryl-cAMP (1 mM; Table 3).

In the rat, the interaction of forskolin with the α_2-adrenoceptor agonist clonidine was tested. When added alone 30 min before S_2, clonidine (0.1 μM) reduced S_2/S_1 to 0.32 ± 0.04 ($n = 4$; $P < 0.001$). A similar ratio was obtained when clonidine (0.1 μM) and forskolin (1 μM) were added simultaneously 30 min before S_2: 0.29 ± 0.03 ($n = 4$). Remember that forskolin alone increased the ratio to 1.30 ± 0.02 ($n = 10$; Table 1).

The chemical composition of the tritiated material in the superfusate was studied in rabbit cortical slices. These experiments were carried out without electrical stimulation. As shown in Fig. 2, the main compound was [^{3}H]-DOPEG; only a small percentage was [^{3}H]-noradrenaline. In control experiments, the outflow of total tritium, [^{3}H]-DOPEG and [^{3}H]-noradrenaline declined continuously. Forskolin (10 μM) and IBMX (1 mM) increased total tritium efflux. The increase was mainly due to [^{3}H]-DOPEG. However, there was also a small increase in [^{3}H]-noradrenaline outflow which preceded the increase in [^{3}H]-DOPEG and total tritium.

Table 2. Effect of IBMX in the presence of forskolin on basal and electrically-evoked tritium efflux from rabbit cerebral cortex slices. Slices were stimulated twice (S_1, S_2). Forskolin (1 μM) was present throughout superfusion, and IBMX was added 25 min before S_2. Values are the ratios of the basal outflows immediately before S_2 and immediately before IBMX addition (b_2/b_1) and of the overflows evoked by S_2 and S_1 (S_2/S_1). Means ± SE of n experiments

Drug added before S_2 (μM)	b_2/b_1	S_2/S_1	n
IBMX			
0	0.92 ± 0.01	0.96 ± 0.01	8
1	1.03 ± 0.01**	1.07 ± 0.03*	4
10	1.08 ± 0.03**	1.39 ± 0.04**	4
100	1.16 ± 0.07**	1.87 ± 0.14**	4
1,000	1.47 ± 0.03**	2.46 ± 0.23**	4

Significant differences from control (IBMX concentration = 0): *$P < 0.05$; **$P < 0.001$

[^3H]-noradrenaline uptake

Within the 15-min period used, the accumulation of tritium in rat and rabbit cortical synaptosomes incubated with [^{3}H]-noradrenaline was linear. Since monoamine oxidase was blocked by pargyline, the accumulation of tritium reflected uptake of [^{3}H]-noradrenaline. The uptake was inhibited by cocaine 0.1 − 100 μM but not changed by forskolin, IBMX and AH21-132 over the same concentration range (Table 5).

cAMP content

In rat cerebral cortex slices, forskolin increased the cAMP content in a concentration-dependent manner (Table 6). At 10 μM, cAMP was increased about 8-fold over control values. Preincubation of the slices with cocaine (10 μM) did not change the effect of forskolin. In contrast, the increase in cAMP caused by noradrenaline was slightly greater in the presence of cocaine (Table 6). IBMX and AH21-132 had little effect on cortical cAMP levels when they were given alone;

Table 3. Effect of dibutyryl-cAMP on basal and electrically-evoked tritium efflux from rat cerebral cortex slices in the absence and in the presence of cocaine. Slices were stimulated twice (S_1, S_2). Cocaine (10 μM), when given, was present throughout superfusion, and dibutyryl-cAMP was added 30 min before S_2. Values are the ratios of the basal outflows immediately before S_2 and immediately before dibutyry--cAMP addition (b_2/b_1) and of the overflows evoked by S_2 and S_1 (S_2/S_1). Means ± SE of n experiments

Drug added before S_2 (μM)	No cocaine			Cocaine (10 μM) throughout		
	b_2/b_1	S_2/S_1	n	b_2/b_1	S_2/S_1	n
dibutyryl-cAMP						
0	0.97 ± 0.03	0.99 ± 0.02	6	0.94 ± 0.03	0.97 ± 0.01	4
10	1.04 ± 0.05	1.08 ± 0.05	3	0.96 ± 0.01	1.00 ± 0.02	3
100	1.05 ± 0.01	1.02 ± 0.03	4	0.97 ± 0.02	0.97 ± 0.04	3
1,000	1.08 ± 0.04	1.16 ± 0.03*	6	1.04 ± 0.04	1.00 ± 0.02	4

Significant difference from control (dibutyryl-cAMP concentration = 0): *$P < 0.001$

Table 4. Effects of forskolin, IBMX and AH21-132 on basal and electrically-evoked tritium efflux from rat and rabbit cerebral cortex slices in the presence of cocaine. Slices were stimulated twice (S_1, S_2). Cocaine (10 μM) was present throughout superfusion, and forskolin, IBMX or AH21-132 was added 30 (rat) or 25 min (rabbit) before S_2. Values are the ratios of the basal outflows immediately before S_2 and immediately before forskolin, IBMX or AH21-132 addition (b_2/b_1) and of the overflows evoked by S_2 and S_1 (S_2/S_1). Means ± SE of n experiments

Drug added before S_2 (μM)	Rat			Rabbit		
	b_2/b_1	S_2/S_1	n	b_2/b_1	S_2/S_1	n
Forskolin						
0	0.93 ± 0.02	0.97 ± 0.02	4	0.78 ± 0.01	1.03 ± 0.01	12
0.01	0.96 ± 0.01	0.99 ± 0.02	4	0.81 ± 0.03	1.04 ± 0.02	6
0.1	0.96 ± 0.02	1.00 ± 0.04	4	0.82 ± 0.01	1.10 ± 0.02*	6
1	1.07 ± 0.02*	1.03 ± 0.03	4	0.86 ± 0.03*	1.17 ± 0.02**	6
10	1.17 ± 0.01**	1.08 ± 0.03*	4	1.04 ± 0.05**	1.24 ± 0.02**	6
IBMX						
0	0.94 ± 0.01	0.96 ± 0.01	8	0.81 ± 0.01	1.02 ± 0.03	12
1	0.96 ± 0.02	0.99 ± 0.02	4	0.84 ± 0.03	1.09 ± 0.02	6
10	0.99 ± 0.02	0.99 ± 0.04	4	0.88 ± 0.03*	1.18 ± 0.03*	6
100	1.02 ± 0.02*	1.03 ± 0.02*	6	0.94 ± 0.02**	1.37 ± 0.05**	6
1,000	1.34 ± 0.06**	1.16 ± 0.05**	4	1.35 ± 0.02**	2.04 ± 0.14**	6
AH21-132						
0	0.93 ± 0.03	0.98 ± 0.04	6	0.78 ± 0.01	1.03 ± 0.03	6
1	0.96 ± 0.02	1.03 ± 0.05	4	0.82 ± 0.01	1.12 ± 0.02	4
10	1.04 ± 0.03*	1.12 ± 0.03*	4	0.91 ± 0.01**	1.48 ± 0.05**	4
100	1.99 ± 0.08**	1.50 ± 0.11*	6	2.04 ± 0.12**	3.08 ± 0.04**	4

Significant differences from control (forskolin, IBMX and AH21-132 concentration = 0): $*P < 0.05$; $**P < 0.001$

when combined with noradrenaline, however, they caused a marked increase (Table 7).

Discussion

Under the conditions of the present experiments, the electrically-evoked overflow of tritium from brain cortex slices preincubated with [^{3}H]-noradrenaline reflects action potential-induced release of [^{3}H]-transmitter from noradrenergic terminal axons (Taube et al. 1977). This release was increased by forskolin, IBMX and AH21-132. The increase was not due to inhibition of the neuronal re-uptake of [^{3}H]-noradrenaline, because neither compound had any effect on the cocaine-sensitive uptake mechanism. It has been suggested that reserpine-like compounds enhance the exocytotic release of noradrenaline by an action on the storage vesicles (Cubeddu and Weiner 1975). Forskolin, IBMX and AH21-132 accelerated the basal outflow of tritium, above all of [^{3}H]-DOPEG (shown for forskolin and IBMX only), and this may reflect a granular action; however, the electrically-evoked overflow was enhanced even under conditions of an unchanged basal outflow (e.g., by IBMX 10 μM; Table 1), indicating that a reserpine-like mechanism is not a sufficient explanation. Finally, forskolin did not counteract the release-inhibiting effect of clonidine, indicating that it did not block the presynaptic α_2-adrenoceptors which both in rat (Hedler et al. 1981) and rabbit brain cortex (Reichenbacher et al. 1982) mediate an autoinhibition of noradrenaline release.

Forskolin activates adenylate cyclase (Metzger and Lindner 1981; Seamon and Daly 1983), and IBMX inhibits PDE. AH21-132 is a new competitive PDE inhibitor; its K_i value for rat whole brain soluble PDE with cAMP as substrate is 6 μM; in contrast to IBMX, it has little effect on adenosine receptors as shown, for instance, by the fact that

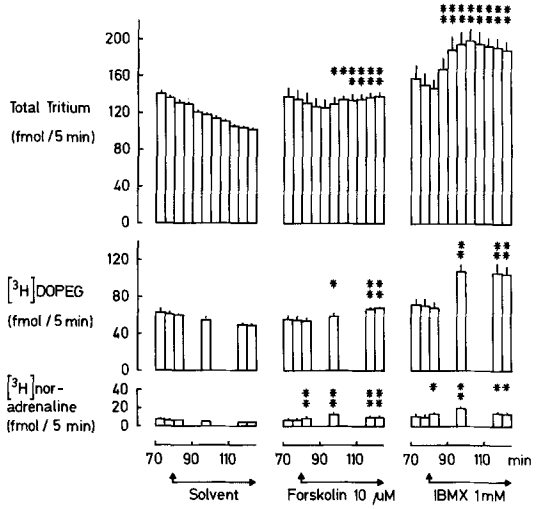

Fig. 2. Outflow of [^{3}H]-compounds from rabbit cerebral cortex slices preincubated with [^{3}H]-noradrenaline, and the effect of forskolin and IBMX. Solvent ($n = 6$), forskolin (10 μM; $n = 3$) or IBMX (1 mM; $n = 3$) was added as indicated. *Abscissa*, min of superfusion; *ordinate*, outflow of total tritium, [^{3}H]-DOPEG and [^{3}H]-noradrenaline (fmol/5 min). [^{3}H]-DOPEG and [^{3}H]-noradrenaline were separated from other [^{3}H]-metabolites only in selected samples. Means ± SE. *Asterisks* indicate significant differences from control ($*P < 0.05$; $**P < 0.001$); the t-test was based on ratios of efflux after solvent or drug addition to efflux immediately before solvent or drug addition (75–80 min of superfusion)

even the high concentration of 100 μM does not inhibit the binding of [^{3}H]-N^6-cyclohexyladenosine to rat brain membranes (Markstein, manuscript in preparation). The following findings suggest that an increase in intraneuronal cAMP is the common mechanism through which forskolin, IBMX and

Table 5. Effects of forskolin, IBMX, AH21-132 and cocaine on the uptake of [^{3}H]-noradrenaline into synaptosomes from rat and rabbit cerebral cortex. The substances were preincubated with the synaptosome preparation for 10 min. [^{3}H]-noradrenaline was then added and the suspension filtered through glass fibre filters 15 min later. For rat and rabbit synaptosomes, the final [^{3}H]-noradrenaline concentration was 3.3 and 2.6 nM, and control [^{3}H]-noradrenaline uptake in excess over blank (0° C) 1 and 4 pmol/g tissue, respectively. Means ± SE of 9 determinations

Test substance concentration (μM)	Synaptosomal [^{3}H]-noradrenaline uptake (% of control)						
	Rat				Rabbit		
	Forskolin	IBMX	AH21-132	Cocaine	Forskolin	IBMX	Cocaine
0.1	103 ± 10	117 ± 13	94 ± 6	83 ± 9	85 ± 7	85 ± 8	73 ± 4
1	94 ± 6	95 ± 12	101 ± 10	54 ± 8*	91 ± 8	90 ± 7	44 ± 8*
10	95 ± 9	93 ± 13	103 ± 10	30 ± 6**	96 ± 10	105 ± 5	20 ± 9**
100	101 ± 12	94 ± 14	102 ± 13	19 ± 4**	100 ± 7	107 ± 6	9 ± 7**

Significant differences from control: $*P < 0.05$; $**P < 0.001$

Table 6. Effects of forskolin and noradrenaline on cAMP content of rat cerebral cortex slices in the absence and in the presence of cocaine. Cocaine (10 μM), when present, was added 15 min before forskolin or noradrenaline. Incubation was continued for 8 min after addition of the latter substances. Means ± SE of 3 determinations

Test substance (μM)	cAMP content (pmol/mg protein)	
	No cocaine	Cocaine (10 μM) present
Forskolin		
0	15.2 ± 1.5	14.5 ± 1.0
0.1	18.3 ± 0.6	17.9 ± 1.2
1	50.3 ± 2.5*	46.6 ± 1.7*
10	113.5 ± 3.9*	112.6 ± 9.8*
Noradrenaline		
0	13.3 ± 0.6	12.9 ± 2.5
1	44.9 ± 1.6*	81.2 ± 3.5*
25	133.6 ± 7.3*	140.1 ± 9.9*
125	142.0 ± 12.4*	143.4 ± 6.1*

Significant differences from control (forskolin and noradrenaline concentration = 0): $*P < 0.001$

Table 7. Effects of IBMX and AH21-132 on cAMP content of rat cerebral cortex slices in the absence and in the presence of noradrenaline. Noradrenaline (1 μM), when given, was added 15 min after IBMX or AH21-132. Incubation was continued for 8 min after addition of noradrenaline. Means ± SE of 3–6 determinations

Test substance (μM)	cAMP content (pmol/mg protein)	
	No noradrenaline	Noradrenaline (1 μM) present
IBMX		
0	13.8 ± 0.9	26.6 ± 1.1
1	14.8 ± 3.0	25.8 ± 3.3
10	12.7 ± 2.3	27.0 ± 1.9
100	13.4 ± 0.9	40.3 ± 1.6**
1,000		70.3 ± 3.5**
AH21-132		
0	13.8 ± 0.9	26.3 ± 2.2
1	13.2 ± 4.0	25.3 ± 0.9
10	14.3 ± 0.6	32.2 ± 1.2
100	22.2 ± 2.7*	53.8 ± 2.7*

Significant differences from control (IBMX and AH21-132 concentration = 0): $*P < 0.05$; $**P < 0.001$

AH21-132 facilitate action potential-induced release of noradrenaline.

1. When submaximal concentrations of forskolin and IBMX were combined, although there was no clear potentiation as has been observed in smooth muscle (Muller and Baer 1983), the effects were at least additive. 2. The cAMP analogue dibutyryl-cAMP also increased the electrically-evoked overflow of tritium, presumably by enhancing the release of noradrenaline. The concentration of dibutyryl-cAMP required (1 mM) was higher than that necessary to enhance the release of noradrenaline in the rat pineal gland (0.1 mM; Pelayo et al. 1978), but similar to concentrations promoting the conversion of tyrosine to dopamine in rat striatal slices (Reisine et al. 1982) or the potassium-evoked release of noradrenaline in rat cortical slices (Wemer et al. 1982). 3. Forskolin, IBMX and AH21-132 all raised the cAMP content of rat cortical slices. The concentrations required tended to be higher than those at which facilitation of noradrenaline release was observed. However, whole slice cAMP levels as in Tables 6 and 7 may not accurately reflect cAMP levels inside the noradrenergic axons. IBMX increased cAMP only, and AH21-132 increased cAMP more markedly, when the PDE inhibitors were combined with noradrenaline. It should be noted that combined exposure to PDE inhibitors and extracellular noradrenaline also occurs in brain slices when they are stimulated electrically in the presence of IBMX or AH21-132.

If cAMP were a necessary link in the sequence leading from membrane depolarization to release, an increase in cAMP should elicit a de novo release (and not only facilitate release triggered by external stimuli). Our results do not exclude this possibility, but argue rather against it. The increase in basal tritium outflow caused by forskolin, IBMX and AH21-132 was small, and that caused by dibutyryl-cAMP not statistically significant. Moreover, the major part of the increase produced by forskolin and IBMX was due to [^{3}H]-DOPEG and not [^{3}H]-noradrenaline – the opposite of the overflow of tritium elicited by electrical stimulation or

high potassium (Taube et al. 1977). Our results are perhaps best explained as Cubeddu et al. (1975) interpreted their experiments on postganglionic sympathetic neurones: cAMP is not indispensible for the release of noradrenaline but facilitates the normal process of release by nerve stimulation.

While our experiments were in progress, three studies of the effect of forskolin on the release of noradrenaline were published. In agreement with our experiments, forskolin 10 μM increased veratrine-induced release of noradrenaline from rat brain cortex slices (Schoffelmeer and Mulder 1983; the authors also showed that electrically-evoked noradrenaline release, in contrast to potassium-evoked release, was enhanced by a PDE inhibitor, again in accord with our results). On the other hand, high concentrations of forskolin (25–150 μM) tended to *reduce* potassium-evoked release of noradrenaline from guinea-pig synaptosomes (Ebstein et al. 1982). Obviously, this is not in contrast to our findings. In fact, in the third study, which was carried out on the PC12 rat pheochromocytoma cell line, the concentration-response curve for enhancement by forskolin of potassium-evoked noradrenaline release was biphasic, with facilitation at forskolin 0.1–10 μM but inhibition at 100 μM (Rabe et al. 1982). The conclusion of these latter authors is the same as ours, namely that "elevation of intracellular cAMP cannot initiate release... but can enhance depolarization-dependent release" of noradrenaline (Rabe et al. 1982).

Unexpectedly, the increase in electrically-evoked tritium overflow produced by forskolin, IBMX, dibutyryl-cAMP and (in the rat only) AH21-132 was inhibited by cocaine. Cocaine did not diminish the forskolin-induced increase in cAMP levels in rat cortical slices (Table 6), and this makes it unlikely that cocaine generally interfered with the formation of cAMP. However, as mentioned above, whole slice cAMP levels need not reflect accurately intraaxonal levels. Cocaine itself greatly increased the evoked overflow of tritium and, presumably, the perineuronal concentration of noradrenaline (Taube et al. 1977). One possible, but of course speculative, reason for the interaction of cocaine with forskolin, IBMX and (in the rat) AH21-132 would be that the increase in cAMP inside the noradrenergic axons is diminished when presynaptic α_2-adrenoceptors are markedly activated by released noradrenaline. Activation of α_2-receptors has in fact been shown to counteract the stimulation of adenylate cyclase by forskolin (Burns et al. 1982; Siegl et al. 1982). However, there are alternative explanations, one being that cocaine somehow interferes with the chain of events connecting the rise in intraneuronal cAMP with facilitation of transmitter release.

Acknowledgements. We thank Professor E. Lindner, Hoechst, for forskolin. This study was supported by the Deutsche Forschungsgemeinschaft (SFB 70).

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Received July 8, 1983/Accepted October 4, 1983