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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*

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Summary. Stimulation-evoked tritium overflow was examined in superfused rat brain cortex slices (stimulus: electrical impulses; 3 Hz) and synaptosomes (stimulus: potassium 12 mmol/l) preincubated with ^{3}H-5-HT. 1. In slices and synaptosomes, the evoked ^{3}H overflow was facilitated by forskolin and 8-Br-cAMP, but was not affected by AH 21-132 (an inhibitor of cAMP phosphodiesterase; cis-6-(p-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy - 2 - methylbenzo [c] [1,6]-naphthyridine). In the presence of AH 21-132, the facilitatory effect of forskolin on evoked overflow was increased. 2. In slices, AH 21-132 or combined administration of forskolin plus AH 21-132 did not change the percentage of basal or evoked ^{3}H overflow represented by unmetabolized ^{3}H-serotonin (about 30% and 60%, respectively). 3. In slices, cocaine or 6-nitroquipazine, an inhibitor of serotonin uptake, did not influence the increase in evoked overflow produced by forskolin plus AH 21-132. Forskolin plus AH 21-132 did not alter the inhibitory effect of serotonin (examined in the presence of 6-nitroquipazine) and the facilitatory effect of metitepin (a serotonin receptor antagonist) on evoked ^{3}H overflow, but considerably decreased the inhibitory effect of clonidine or B-HT 920 (2 - amino - 6 - allyl - 5, 6, 7, 8 - tetrahydro - 4H - thiazolo - [5, 4 - d] - azepine). The present results suggest that the serotoninergic nerve terminals in the rat brain cortex are endowed with an adenylate cyclase, which is negatively coupled to the presynaptic α_2-adrenoceptors, but is not linked to the presynaptic autoreceptors.

Key words: Forskolin – Cyclic AMP – 5-HT release – Presynaptic α_2-adrenoceptors – Presynaptic serotonin autoreceptors

Introduction

Serotonin release in the rat brain cortex appears to be controlled by an adenylate cyclase, since forskolin, an activator of adenylate cyclase (Metzger and Lindner 1981; Seamon and Daly 1981, 1983) has been shown to facilitate the electrically evoked ^{3}H-serotonin release from rat brain cortex slices (Schlicker et al. 1984; Schoffelmeer et al. 1985).

It was the aim of the present study to examine whether the 5-HT nerve terminals themselves are endowed with an adenylate cyclase system. Therefore, not only slices but also synaptosomes were used to determine the effects of forskolin, 8-Br-cAMP and AH 21-132 (an inhibitor of cAMP phosphodiesterase; Markstein et al. 1984) on ^{3}H-5-HT release. Another purpose of the present investigation was to study whether the presynaptic autoreceptors and α_2-adrenoceptors on the serotoninergic nerve terminals (for review see Starke 1981; Göthert 1982, 1985; Moret 1985) are coupled to an adenylate cyclase. Furthermore, the effects of forskolin and AH 21-132 were examined in the presence of 6-nitroquipazine (a selective inhibitor of serotonin uptake in the rat brain: Vaatstra et al. 1981; Classen et al. 1984) and of cocaine, since the latter drug attenuated the facilitatory effect of forskolin on *noradrenaline* release in the rat and rabbit brain cortex (Markstein et al. 1984). Finally, the effects of forskolin and AH 21-132 on the percentage of unmetabolized ^{3}H-serotonin contained in the tritium overflow were investigated.

Methods

Slices and synaptosomes were prepared from the brain cortex of male Wistar rats weighing 200–300 g.

Briefly, occipitoparietal cortex slices (0.3 mm thick, diameter 3 mm) were incubated with 0.1 μmol/l ^{3}H-serotonin (specific activity: 24.1–29.6 Ci/mmol) for 60 min and subsequently superfused with physiological salt solution (for composition, see Classen et al. 1984) for 110 min at a flow rate of 0.5 or 1.0 ml/min (37° C; collection of the superfusate in 5-min samples). Two 2-min periods of electrical field stimulation (rectangular pulses of 3 ms and 20 mA at 3 Hz) were applied to each slice after 40 min and 90 min of superfusion (S_1, S_2).

In some of the experiments, ^{3}H-serotonin was separated from ^{3}H-5-hydroxyindole acetic acid (^{3}H-5-HIAA) as described by Steppeler et al. (1982) with slight modifications. Superfusate samples from slices preincubated with ^{3}H-serotonin 0.2 μmol/l were passed through Dowex 50WX4 columns (0.5 × 1.5 cm; treated as described by Graefe et al. 1973). ^{3}H-Serotonin was eluted with 2 × 1 ml of a mixture of ethanol and HCl 6 mol/l (1:1; v/v). The mean recovery of ^{3}H-serotonin was 95% and that of 5-HIAA (column effluents) was 97% (no cross-contamination); all values were corrected accordingly.

Synaptosomes were prepared according to Mulder et al. (1975) with some modifications. A 10% (w/v) homogenate of cortical tissue (except for the frontal poles) was prepared

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in 0.32 mol/l sucrose in a Potter-Elvehjem glass homogenizer equipped with a rotating teflon pestle (1000 rpm; 2×3 strokes/min). The homogenate was centrifuged at 1000×*g* for 10 min (4° C) and 3 ml of the supernatant were added to 12 ml of physiological salt solution containing pargyline 50 µmol/l (final concentration). The synaptosomes were preincubated for 6 min at 37° C and, after addition of ^{3}H-serotonin (specific activity, see above; final concentration 0.2 µmol/l), the incubation was continued for another 6 min. The labelled particulate material was centrifuged at 600×*g* for 10 min (4° C) and the pellet was resuspended in 1.5 ml ice-cold physiological salt solution. 100 µl-aliquots of this suspension were layered on small columns (300 µl) of Sephadex G 10 (swollen in distilled water) in 12 superfusion chambers (inner diameter 0.51 cm; for further details, see Mulder et al. 1975). Subsequently, synaptosomes were superfused for 60 min at a flow rate of 0.5 ml/min (37° C) and the superfusate was collected in 5-min samples. Tritium overflow was evoked by increasing the concentration of $K^{\oplus}$ to 12 mmol/l (isomolar replacement of NaCl by KCl) from 40–45 min.

At the end of the experiments, the radioactivity of the superfusate samples, slices (solubilized with Soluene) and synaptosomes (extracted with HCl 0.1 mol/l) was determined by liquid scintillation counting.

Tritium efflux from slices and synaptosomes was calculated as the fraction of the ^{3}H content in the slice/synaptosomes at the onset of the respective collection period. In order to quantify drug-induced effects on the basal ^{3}H efflux in synaptosomes, the fractional rate of ^{3}H efflux from 35–40 min was used; in the slices, the ratio of the fractional rate of ^{3}H efflux from 85–90 min (t_2) over that from 55–60 min (t_1) was calculated (t_2/t_1). Stimulation-evoked ^{3}H overflow was calculated by subtraction of the basal from the total efflux during stimulation and the subsequent 13 (slices) or 10 min (synaptosomes) and was expressed as percent of tissue tritium at the onset of stimulation; the basal efflux was assumed to decline linearly from the 5-min period before to that 15–20 min after onset of stimulation. In slices, the ratio of the overflow evoked by S_2 over that evoked by S_1 (S_2/S_1) was calculated.

Means ± SEM of *n* experiments (slices) or, in the case of synaptosomes, of *n* experiments in triplicate are given throughout the paper. For comparison of mean values, Student's *t*-test was used; if more than two values were compared, Bonferroni's procedure was used (Sachs 1984).

Drugs used. 5-[1,2-^{3}H(N)]-Hydroxytryptamine creatinine sulphate (^{3}H-serotonin; NEN, Dreieich, FRG); AH 21-132 (cis-6-(p-acetamidophenyl)-1,2,3,4,4a,10b-hexahydro-8,9-dimethoxy-2-methylbenzo [c] [1,6]-naphthyridine-bis (hydrogenmaleinate); Sandoz, Basel, Switzerland); B-HT 920 (6-allyl-2-amino-5,6,7,8-tetrahydro-4H-thiazolo-[5,4-d]-azepine dihydrochloride; Thomae, Biberach an der Riss, FRG); 8-Br-cAMP (8-bromoadenosine 3′,5′-cyclic monophosphate (sodium salt); Sigma, München, FRG); clonidine hydrochloride (Boehringer Ingelheim, Ingelheim, FRG); cocaine hydrochloride, 5-hydroxytryptamine creatinine sulphate (Merck, Darmstadt, FRG); 7-deacetylforskolin (Farbwerke Hoechst, Frankfurt, FRG); forskolin (Calbiochem-Behring, La Jolla, CA, USA or Farbwerke Hoechst, Frankfurt, FRG); metitepin maleate (Roche, Basel, Switzerland); 6-nitroquipazine (DU 24565; Duphar, Weesp, the Netherlands); pargyline hydrochloride (Abbott, North Chicago, IL, USA). Stock solutions of forskolin were prepared with dimethyl sulphoxide (DMSO) and diluted with physiological salt solution. At the concentrations used in the present study (up to 0.32% v/v), DMSO did not influence basal or evoked ^{3}H overflow.

Results

1. Experiments on slices

Basal tritium efflux. The basal ^{3}H efflux was slightly increased by forskolin 1 and 10 µmol/l, by AH 21-132 10 µmol/l (Table 1), and by combined administration of forskolin 10 µmol/l plus AH 21-132 10 µmol/l (by 25%; result not shown). The basal ^{3}H efflux was also enhanced by metitepin 3.2 µmol/l (both in the absence and presence of forskolin 10 µmol/l plus AH 21-132 10 µmol/l; by 12% and 30%, respectively; results not shown) and by B-HT 920 100 µmol/l (by 19% and 20%, respectively; results not shown).

In control experiments ($n = 9$), 31.7% ± 3.2% of tritium efflux during t_2 consisted of unmetabolized ^{3}H-serotonin; in the presence of AH 21-132 10 µmol/l or of forskolin 10 µmol/l plus AH 21-132 10 µmol/l, 37.6% ± 1.2% and 26.8% ± 1.5% of the tritium efflux during t_2 were accounted for by ^{3}H-serotonin ($n = 9$ and 5; not significantly (n.s.) different from controls).

Electrically evoked ^{3}H overflow. The effects of forskolin, AH 21-132 and 8-Br-cAMP on the electrically evoked ^{3}H overflow were studied in experiments in which 6-nitroquipazine 10 µmol/l was present throughout superfusion. Forskolin 10 µmol/l and 8-Br-cAMP 320 µmol/l increased the evoked overflow, whereas forskolin 1 µmol/l or AH 21-132 1 and 10 µmol/l had no effect (Table 1). The 7-deacetyl analogue of forskolin which was examined in the absence of 6-nitroquipazine produced a concentration-dependent increase in evoked ^{3}H overflow (S_2/S_1 at various concentrations of 7-deacetylforskolin, present 25 min before and during S_2: 0 µmol/l (controls), 1.01 ± 0.07; 1 µmol/l, 1.07 ± 0.05, n.s.; 10 µmol/l, 1.11 ± 0.15, n.s.; 32 µmol/l, 1.34 ± 0.09, $P < 0.05$; $n = 6–9$). In another series of experiments, the effect of combined administration of forskolin and AH 21-132 was examined. AH 21-132 10 µmol/l plus forskolin 10 µmol/l, added to the superfusion medium from 25 min prior to S_2 onward, increased the evoked overflow by 39% (Fig. 1), i.e. more than in experiments in which forskolin was administered alone (compare with Table 1). The facilitatory effect of AH 21-132 10 µmol/l plus forskolin 10 µmol/l on the evoked ^{3}H overflow was not significantly changed by 6-nitroquipazine or cocaine (present throughout superfusion; Fig. 1).

Furthermore, the interaction of forskolin 10 µmol/l plus AH 21-132 10 µmol/l (present throughout superfusion) with drugs acting on presynaptic serotonin autoreceptors or α_2-adrenoceptors (present before and during S_2) was studied. In the experiments with unlabelled serotonin, but not in those with the other receptor agonists and antagonists, 6-nitroquipazine 1 µmol/l was present throughout superfusion. In the absence of forskolin plus AH 21-132, serotonin decreased and the serotonin receptor antagonist metitepin increased the evoked ^{3}H overflow in a concentration-dependent manner (Fig. 2); the α_2-adrenoceptor agonists clonidine and B-HT 920 concentration-dependently

Table 1. Effect of forskolin, AH 21-132, combination of both drugs or of 8-Br-cAMP on the basal and evoked ^3H overflow from superfused rat brain cortex slices (a) and synaptosomes (b) preincubated with ^3H-serotonin. In *slices* (a), the drug under study was present from the 65th min of superfusion onward and 6-nitroquipazine 10 μmol/l throughout superfusion. Tritium overflow was evoked by electrical stimulation (3 Hz) after 40 min and 90 min of superfusion (S_1, S_2), and the ratio of tritium overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). For estimation of the basal efflux, the ratio of the fractional rate of ^3H efflux during t_2 (85–90 min of superfusion) over that during t_1 (55–60 min of superfusion) was determined (t_2/t_1). In *synaptosomes* (b), the drug(s) under study was (were) present from the 25th min of superfusion onward. Tritium overflow was evoked by potassium 12 mmol/l after 40 min of superfusion. Basal efflux: fractional rate of ^3H efflux from 35–40 min of superfusion. Means ± SEM

Drug	Concentration (μmol/l)	*n*	Basal ^3H efflux		Electrically evoked ^3H overflow	
			t_2/t_1	(% of controls)[a]	S_2/S_1	(% of controls)[a]
(a) Slices						
–	–	10	0.83 ± 0.02		1.04 ± 0.03[b]	
Forskolin	1	5	0.97 ± 0.03*	(117)	1.17 ± 0.05	(113)
	10	5	0.98 ± 0.03**	(118)	1.31 ± 0.07*	(126)
AH 21-132	1	5	0.87 ± 0.04	(105)	1.14 ± 0.04	(110)
	10	5	1.00 ± 0.05*	(120)	0.94 ± 0.07	(90)
8-Br-cAMP	320	7	0.89 ± 0.03	(107)	1.23 ± 0.06*	(118)
Drug (s)	Concentration (μmol/l)	*n*	Basal ^3H efflux		Potassium-evoked ^3H overflow	
			% of tissue ^3H/5 min	(% of controls)[a]	% of tissue ^3H	(% of controls)[a]
(b) Synaptosomes						
–	–	6	1.53 ± 0.05		3.68 ± 0.25[c]	
Forskolin	1	6	1.57 ± 0.09	(103)	3.67 ± 0.15	(100)
–	–	6	1.31 ± 0.12		4.67 ± 0.32	
Forskolin	10	6	1.41 ± 0.07	(108)	5.52 ± 0.37**	(118)
–	–	6	1.53 ± 0.05		3.68 ± 0.25	
AH 21-132	1	6	1.48 ± 0.09	(96)	3.88 ± 0.10	(105)
–	–	5	1.27 ± 0.13		5.10 ± 0.17	
AH 21-132	10	5	1.44 ± 0.07	(113)	5.43 ± 0.17	(106)
–	–	8	1.74 ± 0.08		4.79 ± 0.20	
Forskolin AH 21-132	1 plus 1	8	1.72 ± 0.09	(99)	5.33 ± 0.23**	(111)
–	–	7	1.80 ± 0.09		4.59 ± 0.20	
8-Br-cAMP	100	7	1.69 ± 0.04	(94)	5.33 ± 0.18*	(116)

* $P < 0.05$; ** $P < 0.01$ (compared to the corresponding controls)
[a] Mean values
[b] ^3H evoked by S_2: 0.92 ± 0.17 nCi (corresponding to 4.16% ± 0.47% of tissue tritium)
[c] Corresponding to 5.34 ± 0.57 nCi

diminished the evoked overflow (Fig. 3). Forskolin plus AH 21-132 did not affect the concentration-response curves of serotonin and metitepin (Fig. 2), but considerably decreased or even abolished the inhibitory effect of clonidine and B-HT 920 (Fig. 3).

The percentage of unmetabolized ^3H-serotonin contained in the ^3H overflow evoked by S_2 was 59.5% ± 3.9% in 9 control experiments (no drug present before and during S_2) and was not affected by AH 21-132 10 μmol/l (62.9% ± 3.2% of evoked ^3H overflow; $n = 9$) and by forskolin 10 μmol/l plus AH 21-132 10 μmol/l (61.1% ± 5.1% of evoked ^3H overflow; $n = 5$), which were present 25 min before and during S_2.

2. *Experiments on synaptosomes*

The basal ^3H efflux was not affected by the drugs examined (Table 1). The potassium-evoked ^3H overflow was increased by forskolin 10 μmol/l and by 8-Br-cAMP 100 μmol/l, whereas AH 21-132 1 and 10 μmol/l had no effect (Table 1). Forskolin 1 μmol/l was also ineffective, but when administered together with AH 21-132 1 μmol/l it increased the evoked overflow (Table 1).

Discussion

The present study revealed that forskolin (an activator of adenylate cyclase), its 7-deacetyl derivative and 8-Br-cAMP (a membrane-permeable analogue of cAMP) increased the electrically evoked ^3H overflow from rat brain cortex slices. Under the present conditions ^3H overflow reflects transmitter release from serotoninergic nerve terminals (Göthert and Weinheimer 1979; Göthert 1980; Classen et al. 1984). The findings with forskolin and 8-Br-cAMP are in agreement with data reported by Schoffelmeer et al. (1985). In our study, the effect of forskolin was not diminished in the

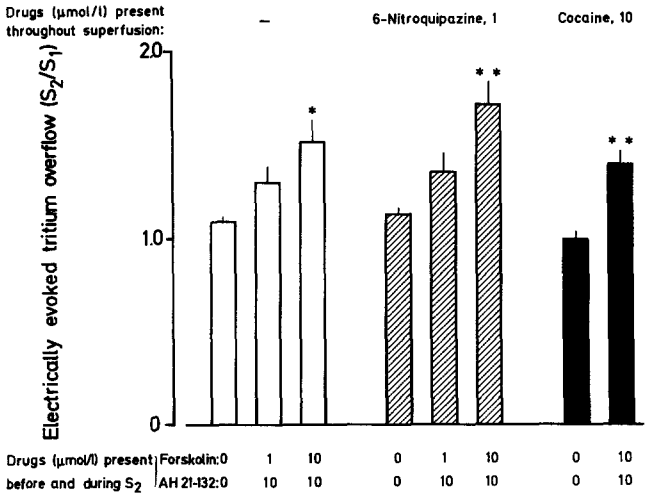

Fig. 1. Effect of forskolin plus AH 21-132 on the electrically evoked ^3H overflow from superfused rat brain cortex slices preincubated with ^3H-serotonin, and interaction with 6-nitroquipazine or cocaine. Forskolin plus AH 21-132 were added to the superfusion medium from the 65th min of superfusion onward whereas cocaine or 6-nitroquipazine was present throughout superfusion. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S_1 and S_2), and the ratio of tritium overflow evoked by S_2 over that evoked by S_1 (S_2/S_1) was determined. In the three series of control experiments, tritium overflow evoked by S_2 was 3.08% ±0.28% of tissue tritium (experiments without interacting drug), 3.82% ± 0.28% (experiments with 6-nitroquipazine) and 2.85% ±0.29% (experiments with cocaine). Means ±SEM of 5–9 experiments. * $P<0.02$, ** $P<0.01$ (compared to the corresponding controls)

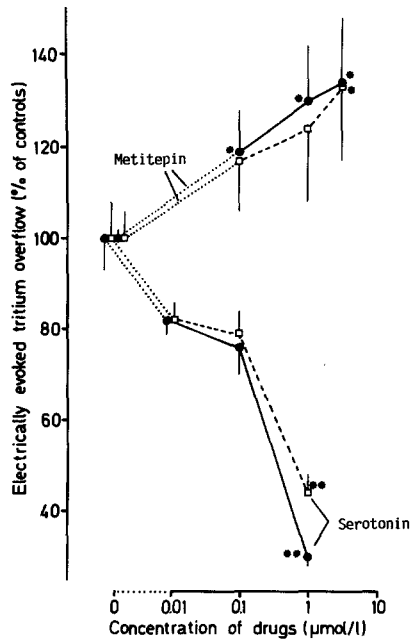

Fig. 2. Effects of unlabelled serotonin and metitepin on the electrically evoked ^3H overflow in the absence (●——●) or presence of forskolin plus AH 21-132 (□- - - -□). Rat brain cortex slices were preincubated with ^3H-serotonin and subsequently superfused. Serotonin or metitepin was added to the superfusion medium from the 65th min of superfusion onward, whereas forskolin 10 µmol/l plus AH 21-132 10 µmol/l and, in the experiments with serotonin, 6-nitroquipazine were present throughout superfusion. Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 (S_2/S_1) was determined. The evoked ^3H overflow is given as percent of the ratio S_2/S_1 in the corresponding controls (serotonin or metitepin absent). The S_2/S_1 values (±SEM) in the four series of control experiments ranged from 0.90 (±0.06) to 1.13 (±0.08). Means ± SEM of 5–18 experiments. * $P<0.05$, ** $P<0.005$ (compared to the corresponding controls)

presence of inhibitors of serotonin and/or noradrenaline uptake; such an attenuation was observed for the facilitatory effect of forskolin on noradrenaline release in the rat and rabbit brain cortex (Markstein et al. 1984), but not in the rabbit pulmonary artery (Göthert and Hentrich 1984). Furthermore, we found that forskolin and 8-Br-cAMP facilitated the potassium-evoked ^3H overflow from synaptosomes as well; in this preparation ^3H overflow may also be assumed to reflect 5-HT release from serotoninergic nerve terminals (Mulder et al. 1975; own unpublished results). AH 21-132, a selective inhibitor of cAMP phosphodiesterase devoid of antagonistic properties at adenosine receptors (Markstein et al. 1984), failed to influence the evoked overflow both in slices and synaptosomes; this finding confirms data obtained in slices with rolipram (ZK 62 711), another inhibitor of phosphodiesterase (Schoffelmeer et al. 1985).

The ability of forskolin and 8-Br-cAMP to increase ^3H-5-HT release in synaptosomes, i.e. resealed torn-off axon terminals, provides evidence that these drugs act on the serotoninergic nerve terminals themselves rather than on interneurones. In addition, several findings support the idea that the release-facilitating effect of forskolin is due to an increase in cAMP levels in the 5-HT nerve terminals:

First, a series of mechanisms known to increase the evoked overflow could be excluded. Thus, forskolin appears not to be an antagonist at the presynaptic 5-HT autoreceptor, since in the presence of forskolin plus AH 21-132 the effect of the well-characterized 5-HT autoreceptor antagonist metitepin was not attenuated. Furthermore, unlike 5-HT autoreceptor antagonists, forskolin also acted facilitatory in synaptosomes, i.e. in a condition in which released 5-HT is immediately removed by the superfusion stream. The same reason argues against the possibility that an inhibition of 5-HT uptake accounts for the facilitatory effect of forskolin on the evoked overflow. An inhibition of 5-HT uptake is also excluded by the finding that in slices 6-nitroquipazine, an inhibitor of serotonin uptake, did not abolish the facilitatory effect of forskolin (in the presence of AH 21-132) on ^3H-5-HT overflow. The increase in 5-HT release by forskolin can also not be attributed to an inhibition of monoamine oxidase, since forskolin (in the presence of AH 21-132) did not affect the proportion of tritium overflow accounted for by unmetabolized ^3H-5-HT.

Second, the potency ratio of forskolin and its 7-deacetyl analogue to activate adenylate cyclase (Seamon et al. 1984), to inhibit ^3H-forskolin binding (Seamon et al. 1984) and to facilitate the evoked overflow (present results) was similar in the three systems: 7-deacetylforskolin was by about one log unit less potent than forskolin itself. In addition, 8-Br-cAMP, a membrane-permeable analogue of cAMP, mimicked the effect of forskolin on the evoked ^3H overflow. Furthermore, forskolin facilitated the evoked overflow in the same concentration range, in which its facilitatory effect on the synthesis of cAMP occurred (Markstein et al. 1984). Finally, and perhaps most important, low concentrations of forskolin, which, given alone, failed to influence the evoked

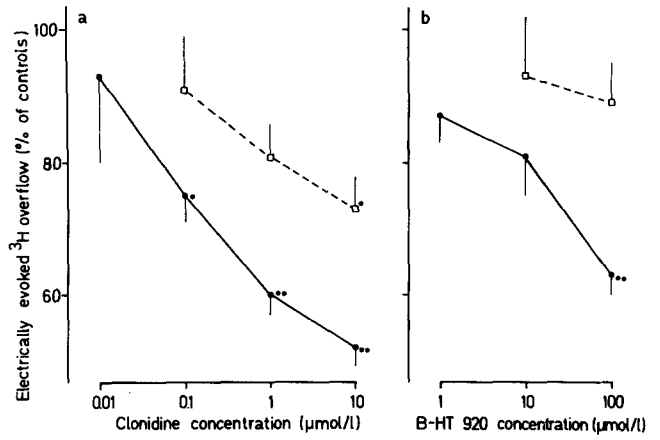

Fig. 3. Effects of clonidine (**a**) and B-HT 920 (**b**) on the electrically evoked ^{3}H overflow in the absence (●——●) or presence of forskolin plus AH 21-132 (□----□). Rat brain cortex slices were preincubated with ^{3}H-serotonin and subsequently superfused. Clonidine or B-HT 920 was present in the superfusion fluid from the 65th min of superfusion onward whereas forskolin 10 μmol/l plus AH 21-132 10 μmol/l were present throughout superfusion. Tritium overflow was evoked twice, after 40 min and 90 min of superfusion (S_1 and S_2), and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1). The evoked ^{3}H overflow is given as percent of the ratio S_2/S_1 in the corresponding controls (clonidine or B-HT 920 absent). The S_2/S_1 values (±SEM) in the four series of control experiments ranged from 0.76 (±0.05) to 1.07 (±0.05). Means±SEM of 5–9 experiments. * $P<0.05$; ** $P<0.001$ (compared to the corresponding controls)

overflow, increased it in the presence of AH 21-132 (present study).

One may conclude from the results discussed so far that serotoninergic nerve terminals are endowed with an adenylate cyclase, activation of which, by increasing intraneuronal cAMP, facilitates stimulation-evoked ^{3}H-5-HT release. Therefore, the question arises whether this adenylate cyclase may be coupled to presynaptic receptors. In this respect, two types of release-inhibiting presynaptic receptors, namely 5-HT autoreceptors (for reviews, see Göthert 1982, 1985; Moret 1985) and α_2-adrenoceptors (for reviews, see Starke 1981; Göthert 1982), were considered in the present study, in which the interaction of agonists and/or antagonists of these receptors with forskolin plus AH 21-132 was examined.

The inhibitory effect of the α_2-adrenoceptor agonists clonidine and B-HT 920 on the evoked overflow was strongly attenuated by forskolin plus AH 21-132. This attenuation cannot be related to blockade of the presynaptic α_2-adrenoceptors by forskolin and/or AH 21-132 since both drugs do not possess antagonistic properties at these receptors (Göthert and Hentrich 1984; Markstein et al. 1984). It rather suggests that the presynaptic α_2-adrenoceptors are negatively coupled to the adenylate cyclase mentioned above. Accordingly, activation of the α_2-adrenoceptors may produce an inhibition of the enzyme, and the resulting decrease in intraneuronal cAMP probably accounts for the diminished transmitter release. A negative coupling of α_2-adrenoceptors to adenylate cyclase has been demonstrated in many tissues (for review, see Exton 1985; Limbird and Sweatt 1985) and has recently also been shown for presynaptic α_2-adrenoceptors inhibiting noradrenaline release in the rat brain (for reviews, see Mulder et al. 1984; Mulder and Schoffelmeer 1985).

The autoreceptor-mediated inhibitory effect of 5-HT and the facilitatory effect of the 5-HT receptor antagonist metitepin were not influenced by forskolin plus AH 21-132. Hence, the serotonin autoreceptor which is of the 5-HT_{1B} subtype (Engel et al. 1986) does not appear to be coupled to an adenylate cyclase. In agreement with this, 5-HT_{1A}, but not 5-HT_{1B}, binding sites were found to be linked to an adenylate cyclase (De Vivo and Maayani 1986; Markstein et al. 1986).

In this context it is of interest to note that presynaptic prostaglandin E receptors on 5-HT neurones do also not appear to be coupled to an adenylate cyclase (Schlicker et al. 1987).

In conclusion, the facilitation of evoked 5-HT release by forskolin may be attributed to an increase in cAMP levels which in turn is due to activation of an adenylate cyclase in the serotoninergic nerve terminals themselves. The inhibitory presynaptic α_2-adrenoceptors, unlike the presynaptic 5-HT autoreceptors and prostaglandin E receptors, appear to be negatively coupled to this adenylate cyclase.

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