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Short communication

Cyclic AMP facilitates the electrically evoked release of radiolabelled noradrenaline, dopamine and 5-hydroxytryptamine from rat brain slices

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Summary. The adenylate cyclase activator forskolin as well as 8-bromo-cyclic AMP enhanced the electrically evoked release of ^{3}H-noradrenaline and ^{3}H-5-hydroxytryptamine from superfused rat neocortical slices and that of ^{3}H-dopamine from neostriatal slices with comparable EC_{50}'s of about 0.5 and 50 μM, respectively, without affecting spontaneous tritium efflux. The phosphodiesterase inhibitor ZK 62771 (3–100 μM) also enhanced ^{3}H-noradrenaline and ^{3}H-dopamine release but slightly reduced ^{3}H-5-hydroxytryptamine release. However, this drug profoundly enhanced spontaneous tritium release in the latter case. The facilitatory effect of forskolin (0.3 μM) on the release of the amine neurotransmitters was potentiated in the presence of ZK 62771 (30 μM). Therefore, cyclic AMP appears to exert a general facilitatory effect on the release of these biogenic amines from central nerve terminals.

Key words: Noradrenaline release – Dopamine release – 5-Hydroxytryptamine release – Cyclic AMP – Brain slices

Introduction

Recent studies from our own and other laboratories have demonstrated that various drugs which are known to enhance intracellular cyclic AMP levels facilitate the depolarization-induced release of ^{3}H-noradrenaline from peripheral tissues (Pelayo et al. 1978; Stjärne et al. 1979) and from brain slices (Wemer et al. 1982; Schoffelmeer and Mulder 1983; Markstein et al. 1984). Accordingly, membrane permeating analogues of cyclic AMP and the adenylate cyclase activator forskolin facilitated ^{3}H-noradrenaline release. However, the effects of phosphodiesterase inhibitors, e.g. ZK 62771, were shown to be dependent on the stimulus used to evoke neurotransmitter release, i.e. the electrically evoked release of ^{3}H-noradrenaline from rat neocortex slices was enhanced while that induced by high K^+ and veratrine was reduced (Wemer et al. 1982; Schoffelmeer and Mulder 1983).

Conflicting data have been reported with respect to the possible role of cyclic AMP as a modulator of the release of dopamine from brain slices. For example, it has been reported that dibutyryl-cyclic AMP enhanced the electrically evoked release of ^{3}H-dopamine from rat neostriatal slices (Westfall et al. 1976), whereas in other studies (Patrick and Barchas 1976; Reisine et al. 1982) no effect of this membrane-permeating cyclic AMP analogue on veratridine-induced and spontaneous release of dopamine was observed. Furthermore, the possibility that cyclic nucleotides modulate the release of 5-hydroxytryptamine from brain tissue still remains to be investigated. Therefore, in a comparative study we examined the effect of increasing the intracellular cyclic AMP levels on the electrically evoked release of the radiolabelled amine neurotransmitters from rat brain slices.

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Materials and methods

Male Wistar rats (140–180 g body weight) were decapitated and the neocortex and neostriatum were rapidly dissected from the brain. Slices (0.3 × 0.3 × 2 mm) were prepared using a McIlwain tissue chopper. The slices (100 mg of fresh tissue weight) were incubated in 5 ml of Krebs-Ringer bicarbonate medium, containing 0.05 μM ^{3}H-noradrenaline or 0.05 μM ^{3}H-5-hydroxytryptamine (neocortical slices) or 0.1 μM ^{3}H-dopamine (neostriatal slices). The composition of the medium was (in mM): NaCl (118), KCl (1.85), KH_2PO_4 (1.15), $MgSO_4$ (1.15), $CaCl_2$ (1.2), $NaHCO_3$ (25), glucose (11.1), gassed with 95% O_2-5% CO_2; pH 7.2–7.4. After labelling the medium was decanted and slices were transferred to each of 24 chambers (about 4 mg of tissue per chamber; 0.20 ml volume) of a superfusion apparatus and subsequently superfused (0.25 ml/min) with medium (gassed with 95% O_2-5% CO_2) at 37°C. After 30 min of superfusion the superfusate was collected in 10-min samples. During superfusion, calcium-dependent neurotransmitter release was induced by electrical square-wave pulses of biphasic polarity and 4 ms duration (^{3}H-noradrenaline release: 1 Hz, 12 mA; ^{3}H-dopamine and ^{3}H-5-hydroxytryptamine release: 3 Hz, 24 mA) at 50 min after the onset of superfusion (t = 50 min) for 5 min. Drugs were added to the medium 20 min prior to stimulation. At the end of the experiment, the remaining radioactivity was extracted from the tissue with 0.1 N HCl. The radioactivity in superfusion fractions and tissue extracts was determined by liquid-scintillation counting.

Tritium efflux during each collection period was expressed as the percentage of the amount of tritium in the slices at the beginning of the respective 10-min collection period. In order to calculate electrically evoked ^{3}H-amine release the spontaneous efflux of radioactivity was subtracted from the total overflow of radioactivity during stimulation and the following 15 min. The spontaneous tritium efflux was calculated, assuming a linear decline from

Table 1. Effects of forskolin, 8-bromo-cyclic AMP and ZK 62771 on the electrically evoked tritium release from rat brain slices labelled with ^{3}H-noradrenaline, ^{3}H-5-hydroxytryptamine (neocortical slices) or ^{3}H-dopamine (neostriatal slices). After labelling slices were superfused and stimulated electrically at $t = 50$ min for 5 min. Drugs were added 20 min prior to stimulation. Basal tritium efflux from slices labelled with ^{3}H-noradrenaline and ^{3}H-dopamine amounted to 2.31 ± 0.04 and 2.47 ± 0.06% of total tissue tritium, respectively, and was not affected by the drugs. Basal tritium efflux from slices labelled with ^{3}H-5-hydroxytryptamine amounted to 3.94 ± 0.06% of total tissue tritium and was not affected by forskolin and 8-bromo-cyclic AMP, but enhanced to 4.12 ± 0.08, 4.41 ± 0.06, 4.82 ± 0.09 and 5.01 ± 0.13% of total tissue tritium by 3, 10, 30 and 100 μM ZK 62771, respectively. Data represent means ± SEM of 6–12 observations

Drugs (μM)		^{3}H-amine release (% of total tissue tritium)		
		^{3}H-noradrenaline	^{3}H-5-hydroxytryptamine	^{3}H-dopamine
Forskolin	0	3.04 ± 0.08	3.28 ± 0.04	2.51 ± 0.10
	0.3	3.51 ± 0.04*	3.48 ± 0.12	2.69 ± 0.07
	1	4.31 ± 0.06*	4.32 ± 0.08*	3.01 ± 0.05*
	10	4.52 ± 0.09*	4.62 ± 0.11*	3.20 ± 0.08*
	30	4.48 ± 0.10*	4.74 ± 0.10*	3.24 ± 0.05*
8-Bromo-cyclic AMP	0	2.91 ± 0.07	3.17 ± 0.14	2.22 ± 0.09
	30	3.42 ± 0.05*	3.42 ± 0.13	2.38 ± 0.12
	100	4.01 ± 0.08*	4.28 ± 0.09*	2.75 ± 0.06*
	300	4.39 ± 0.10*	4.56 ± 0.21*	2.96 ± 0.04*
	1000	4.28 ± 0.09*	4.59 ± 0.09*	2.99 ± 0.06*
ZK 62771	0	2.94 ± 0.07	3.30 ± 0.10	2.34 ± 0.09
	3	3.18 ± 0.15	3.24 ± 0.08	2.44 ± 0.04
	10	3.58 ± 0.11*	3.01 ± 0.07	2.72 ± 0.06*
	30	3.96 ± 0.08*	2.82 ± 0.09	2.90 ± 0.04*
	100	3.98 ± 0.11*	2.72 ± 0.13	2.93 ± 0.12*

* Significantly higher than found in the absence of drugs ($P < 0.02$)

Table 2. Effects of a low concentration of forskolin on the electrically evoked release of ^{3}H-noradrenaline, ^{3}H-5-hydroxytryptamine (neocortical slices) and ^{3}H-dopamine (neostriatal slices) in the absence and presence of ZK 62771. Slices prelabelled with the radioactive amine were superfused and stimulated electrically at $t = 50$ min for 5 min. Drugs were added 20 min prior to stimulation. Control ^{3}H-noradrenaline, ^{3}H-5-hydroxytryptamine and ^{3}H-dopamine release, i.e. in the absence of drugs, amounted to 2.86 ± 0.08, 3.41 ± 0.07 and 2.29 ± 0.04% of total tissue tritium, respectively. Data represent means ± SEM of 6 observations

Drugs	^{3}H-amine release (% of control)		
	^{3}H-noradrenaline	^{3}H-5-hydroxytryptamine	^{3}H-dopamine
None	100 ± 3	100 ± 2	100 ± 2
0.3 μM forskolin	115 ± 4	108 ± 2	107 ± 3
30 μM ZK 62771	127 ± 3*	88 ± 4	121 ± 3*
0.3 μM forskolin + 30 μM ZK 62771	162 ± 5*	119 ± 3*	148 ± 5*

* Significantly different from the sum of the effects of forskolin and ZK 62771 alone ($P < 0.05$)

the 10-min interval before to that 20–30 min after the onset of stimulation. Then the evoked release was expressed as percent of the tritium content of the slices at the start of the stimulation period. Statistical evaluation was carried out with the two-tailed Student's *t*-test.

1-[7,8]-^{3}H-noradrenaline hydrochloride (32 Ci/mmol) and [7,8-^{3}H]-dopamine hydrochloride (40 Ci/mmol) were obtained from the Radiochemical Centre (Amersham) and [1,2-^{3}H]-5-hydroxytryptamine creatinine sulfate (27.4 Ci/mmol) from New England Nuclear. 8-Bromo-cyclic AMP was obtained commercially (Sigma, St. Louis, MO, USA). Forskolin and 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrolidone (ZK 62771) were kindly donated by Dr. J. W. Daly (National Institutes of Health, Bethesda, USA) and Schering AG, respectively.

Results

In the absence of drugs the electrically evoked release of ^{3}H-noradrenaline and ^{3}H-5-hydroxytryptamine from neocortical slices amounted to about 3% of total tissue tritium, whereas a slightly lower percentage was released in case of ^{3}H-dopamine release from neostriatal slices (Table 1). Forskolin and 8-bromo-cyclic AMP both enhanced the electrically evoked release of the radiolabelled amine neurotransmitters with EC_{50}'s of approximately 0.5 and 50 μM, respectively, independent of the ^{3}H-amine studied. ZK 62771 also concentration-dependently facilitated the evoked release of ^{3}H-noradrenaline and ^{3}H-dopamine, but ^{3}H-5-hydroxytryptamine release was slightly lowered. At the concentrations studied, ZK 62771 enhanced the spontaneous efflux of tritium from ^{3}H-5-hydroxytryptamine-labelled slices only (see legend to Table 1). Table 2 shows that the facilitatory effects of forskolin (0.3 μM) and ZK 62771 (30 μM) on the release of ^{3}H-noradrenaline and ^{3}H-dopamine were more than additive. Moreover, the facilitatory effect of forskolin on ^{3}H-5-hydroxytryptamine release was enhanced in the presence of ZK 62771.

Discussion

The electrically evoked release of tritium from rat brain slices, incubated with radioactive biogenic amines, resembles action potential-induced neurotransmitter release in that in vitro release is completely dependent on extracellular Ca^{2+}

and blocked by the Na^+ channel blocker tetrodotoxin (Mulder 1982). The present study shows that, under carefully controlled experimental conditions, i.e. when a similar, low percentage of the different (radiolabelled) biogenic amines is released from brain slices by electrical field stimulation, an increase in the intracellular cyclic AMP levels facilitates neurotransmitter release.

Accordingly, the diterpene forskolin which strongly activates adenylate cyclase in rat neocortical slices (Daly et al. 1982) substantially enhanced the electrically evoked release of ^{3}H-noradrenaline and ^{3}H-5-hydroxytryptamine from rat neocortical slices as well as ^{3}H-dopamine from neostriatal slices. Moreover, the membrane permeating cyclic AMP analogue 8-bromo-cyclic AMP similarly facilitated the release of these radiolabelled amines. The potency of these drugs appeared to be independent of the neurotransmitter involved. The effect of cyclic AMP on the release of biogenic amines in the central nervous system may be due to a variety of actions of the nucleotide in nerve terminals. Firstly, cyclic AMP is known to mediate phosphorylation of voltage-sensitive Ca^{2+}-channels enhancing their selective conductance for Ca^{2+} ions (Reuter 1983). Secondly, phosphorylation of intracellular proteins by cyclic AMP, some of which are located in synaptic vesicles, may act in parallel or synergistically with phosphorylation mediated by Ca^{2+} and calmodulin to facilitate the exocytotic process (Nestler and Greengard 1983). Thirdly, in synaptic vesicles and the nerve terminal membrane cyclic AMP and Ca^{2+} have been shown to regulate phospholipase A_2 activity, which might be required for the interaction of the amine-containing vesicles with the plasma membrane followed by fusion and release (Moskowitz et al. 1983). Although our study strongly suggests that an increase in the intraneuronal cyclic AMP levels, e.g. as a consequence of depolarization (Shimizu et al. 1973), may facilitate the exocytotic release of all major biogenic amine neurotransmitters, the data obtained in experiments examining the effects of phosphodiesterase inhibition on release do not completely fit into this picture. Thus, although the specific cyclic AMP phosphodiesterase inhibitor ZK 62771 (Schwabe et al. 1976) significantly enhanced ^{3}H-noradrenaline and ^{3}H-dopamine release in excess of spontaneous efflux, it slightly inhibited ^{3}H-5-hydroxytryptamine release. The latter effect cannot be interpreted unambiguously, since in this case the drug substantially enhanced spontaneous tritium efflux. Nonetheless, the observation that ZK 62771 enhanced the facilitatory effects of a low concentration of forskolin on the release of the biogenic amines studied is consistent with its inhibitory effect on cyclic AMP phosphodiesterase activity. Recent experiments have demonstrated that not only the release of ^{3}H-noradrenaline from neocortical slices but also that from synaptosomes (pinched-off nerve terminals) is strongly facilitated by the drugs used in the present study (Schoffelmeer et al. 1985). These data support the view that cyclic AMP, produced by an adenylate cyclase system localized within noradrenergic nerve terminals, facilitates the process of noradrenaline release. Thus, provided that the regulatory mechanisms involved in the release processed are basically similar in aminergic nerve terminals, irrespective of the neurotransmitter involved, the lack of a facilitatory effect of ZK 62771 on ^{3}H-5-hydroxytryptamine release (possibly as a result of its effect on spontaneous tritium efflux) might be due to an unknown action of this drug unrelated to inhibition of cyclic AMP phosphodiesterase. Obviously, the elucidation of this point awaits the development of more specific phosphodiesterase inhibitors devoid of the seemingly anomalous effects observed in previous studies (Wemer et al. 1982; Schoffelmeer and Mulder 1983) and the present experiments.

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