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CYCLIC AMP-INDUCED RELEASE OF [^{14}C]TAURINE FROM PINEALOCYTES

G.H.T. Wheler and D.C. Klein

Section on Neuroendocrinology
Laboratory of Developmental Neurobiology
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, Maryland 20205

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SUMMARY: Pinealocytes were prelabelled with [^{14}C]taurine. Twenty-four hours later they were treated with derivatives of cyclic AMP. It was found that dibutyryl cyclic AMP and p-chloro-phenyl-thio cyclic AMP treatment caused a large increase in the release of [^{14}C]taurine. The effect of dibutryl cyclic AMP on [^{14}C]taurine release was near maximal fifteen minutes after treatment started. In view of the known stimulatory effects of norepinephrine on pineal cyclic AMP and the recent discovery that norepinephrine causes the release of taurine from pinealocytes, one can conclude that norepinephrine stimulates [^{14}C]taurine release from pinealocytes by acting through a cyclic AMP mechanism.

INTRODUCTION

Taurine occurs in the rat pineal gland at concentrations of about 20 mM, and is apparently located entirely in postsynaptic structures (1,2). We have recently found that [^{14}C]taurine is rapidly released when denervated pineal glands are treated in organ culture with norepinephrine (3), the neurotransmitter of pineal nerves. One other rapid effect of norepinephrine on the pinealocyte is to increase cyclic AMP (4,5). This second messenger controls the activity of N-acetyltransferase, the enzyme which regulates large changes in pineal indoleamine metabolism (7-9). In the present report we have investigated the question of whether cyclic AMP may also stimulate the release of taurine.

MATERIALS AND METHODS

Sprague-Dawley male rats, 80-100 g, were used. Chemicals were obtained commercially.

Pineal glands were cultured for a total period of 48 to 54 hours as previously described (1). During the first 24 hours the glands were incubated in 0.25 mM [^{14}C]taurine(5.04 Ci/mol)-containing culture medium. Culture medium was then replaced with taurine-free medium and glands were incubated under control conditions for a second 24-hour period. During this 48-hour culture period pineal nerves degenerate.

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To test the effects of drugs, glands were transferred into medium containing test compounds after the first 48 hours of culture. The test period was no longer than 8 hours. At the end of the incubation period, glands were removed and homogenized in 0.1 M sodium phosphate buffer, pH 6.8. Samples of culture medium were analyzed for total radioactivity, and glands were analyzed for N-acetyltransferase activity.

Thin-layer chromatographic analysis indicated that all the radioactivity released into the medium migrated with authentic taurine. The four chromatographic systems used were (taurine R_f values appear in parentheses): n-propanol:H_2O, 1:1 (0.5); n-propanol:NH_4OH, 7:3 (0.3); isopropanol:methyl acetate:NH_4OH, 7:9:4 (0.12); n-butanol:acetic acid:H_2O, 12:3:5 (0.2).

RESULTS

[^{14}C]Taurine was released by pineal glands under control conditions, as previously observed (3). Dibutyryl cyclic AMP treatment increased release two-fold (Fig. 1). This effect was not detectable at 0.1 mM dibutyryl cyclic AMP, but was detectable at a concentration of 1 mM. In the same experiment (unpublished data) it was found that this effect of dibutyryl cyclic AMP (1 mM) was about the same as that seen with 1 μM norepinephrine. The effects of dibutyryl cyclic AMP on serotonin N-acetyltransferase activity were also examined (Fig. 1), and it was found that the similar doses of dibutyryl cyclic AMP were required to produce apparent half maximal stimulation of both the enzyme and the [^{14}C]taurine release.

Another derivative of cyclic AMP, ρ-chloro-phenyl-thio cyclic AMP, was found to be a potent stimulator of taurine release (Table 1), indicating butyric acid was not the active agent. In contrast, cyclic AMP was essentially inactive in stimulating taurine release and weakly active in elevating N-acetyltransferase activity. The relative ineffectiveness of cyclic AMP in stimulating N-acetyltransferase, which is probably a result of degredation and poor penetration through cell membranes, has been previously reported (9,10). Finally, it was found that adenosine treatment did not stimulate taurine release or N-acetyltransferase activity (Table 1).

In two cases, 0.01 mM ρ-chloro-phenyl-thio cyclic AMP and 10 mM cyclic AMP, N-acetyltransferase activity was submaximally elevated (less than 25% of maximal stimulation) but [^{14}C]taurine release was not increased. A reasonable expla-

Table 1. Effects of cyclic AMP and related compounds on taurine release and on N-acetyltransferase activity.

Experiment	Test Compound[1]	Concentration (μM)	Medium [^{14}C]taurine (nCi/gland)	N-Acetyltransferase activity (nmol/gland/hr)
I	None		13.9 ± 2.8	0.19 ± 0.06
	pCl ϕSH cAMP	10	14.7 ± 0.5	0.46 ± 0.09[2]
		100	21.2 ± 0.2[2]	6.83 ± 1.35[2]
		1000	31.8 ± 0.3[2]	10.74 ± 1.94[2]
II	None		15.7 ± 1.2[2]	0.02 ± 0.01[2]
	L-Norepinephrine	0.1	28.4 ± 4.3[2]	6.62 ± 0.61[2]
	Cyclic AMP	10,000	16.4 ± 1.2	1.09 ± 0.16[2]
	Adenosine	100	13.6 ± 0.8	0.08 ± 0.03
		1000	19.4 ± 1.3	0.02 ± 0.01

Pineal glands, preincubated with culture medium containing 0.25 mM [^{14}C]taurine, were treated in organ culture with the chemical listed below for a four hour test period. Values are mean ± SE for six glands or three media.

[1]The abbreviation used is pCl ϕSH cAMP, p-chloro-phenyl-thio cyclic AMP;
[2]Significantly different from control values ($p<0.05$).

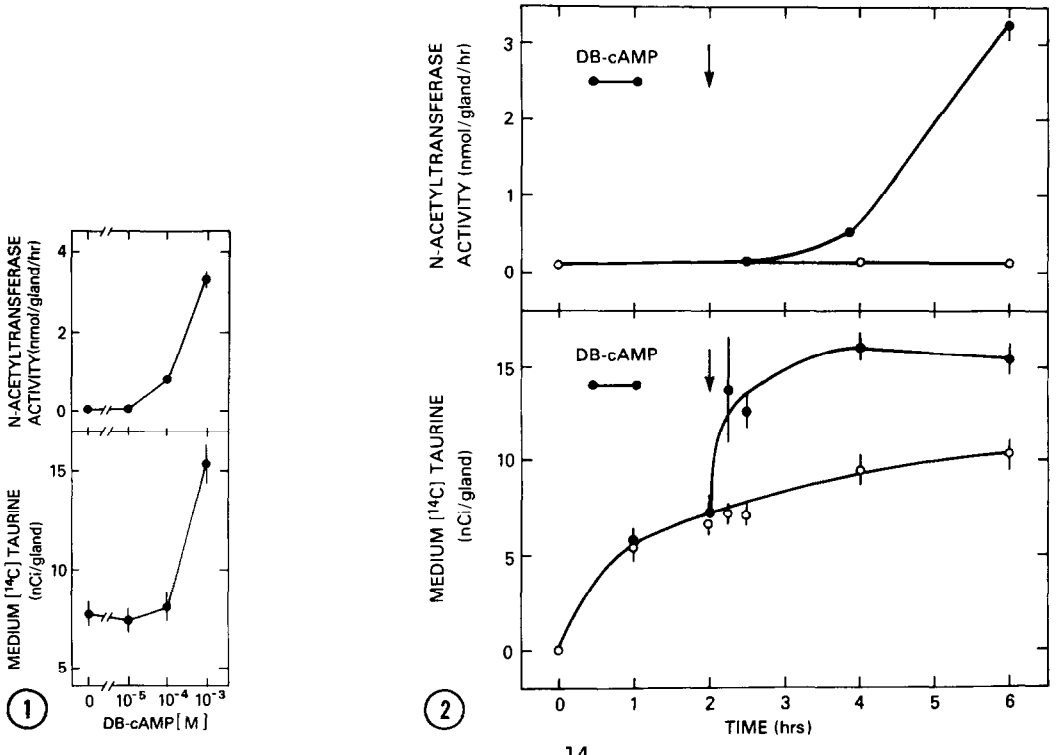

Figure 1. Dose response relationships of [^{14}C]taurine release and of N-acetyltransferase activity with dibutyryl cyclic AMP (DB-cAMP). Dibutyryl cyclic AMP

nation of this apparent discrepancy is that a submaximal increase in [^{14}C]taurine release was undetectable because of re-uptake of released [^{14}C]taurine (2,11,12).

The time course of the effect of dibutyryl cyclic AMP was examined (Figure 2). Near-maximal [^{14}C]taurine release was evident fifteen minutes after treatment had started, and was sustained for 6 hours. The time course for the effect of dibutyryl cyclic AMP on N-acetyltransferase activity is also presented.

DISCUSSION

Our purpose in performing these studies was to determine whether cyclic AMP might mediate the effect of norepinephrine on [^{14}C]taurine release from pinealocytes. The evidence available provided some support for this. First, it was known that norepinephrine could stimulate cyclic AMP, with peak values occurring within 20 minutes of treatment (4,5,9). Second, it was known that the stimulation of taurine release by norepinephrine was near maximal within 15 minutes of treatment (3). The evidence from the experiments presented in this report adds additional support. First, it was found that two cyclic AMP derivatives could stimulate [^{14}C]taurine release. Second, near maximal effects of dibutyryl cyclic AMP were easily detectable within 30 minutes. The possibility that dibutyryl cyclic AMP is acting on presynaptic structures seems remote because during the 48 hour culture period preceeding drug treatment, presynaptic structures disintegrate. Thus, the observations in this report and previous findings provide convincing evidence that cyclic AMP does act as the second messenger mediating the effects of norepinephrine on [^{14}C]taurine release.

Two questions immediately arise regarding this. First, how is cyclic AMP acting? One possibility is that it acts *via* a phosphorylation mechanism to

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was present for the entire four hour test period. Values are the means (± SE) for six glands or three media at four hours.

Figure 2. Time course of dibutyryl cyclic AMP stimulated [^{14}C]taurine release and N-acetyltransferase activity. Glands were incubated for two hours in control medium. Dibutyryl cyclic AMP (final concentration 1 mM) was added in 15 ul of 10x concentrated solution and samples of medium were removed at the times indicated. Diluent was added to control cultures. Several sets of glands were used to prevent removal of more than a total of 30 ul from each dish. Values are the means (±SE) for six media at each time point.

activate a mechanism in the pineal cell membrane mediating the release of taurine. Although cyclic AMP is known to cause phosphorylation of membrane proteins in other neural tissue (12), and phosphorylation of nuclear protein in the pineal gland (8,13), no direct evidence is available at this time to indicate that phosphorylation of membrane proteins in the pineal is involved in the release of taurine. This does, however, seem to be a reasonable hypothesis to pursue.

The second question which arises is why is taurine released? Answers to this question are no more definitive than those to the first question. Taurine is an interesting compound in that the sulfonate group infers unique characteristics. It makes taurine a highly hydrated compound, capable of carrying cations. Perhaps taurine release is a means of transporting ions out of the cell, and that this, in turn, is intimately involved with the norepinephrine-induced hyperpolarization of pineal membranes (14). A second possibility is that the released taurine is acting as an extracellular messenger in the pineal gland. We have previously found that high concentrations of taurine can mimick the effects of norepinephrine on the pineal gland by direct interaction with membrane bound β-receptors (1). Based on this apparent ability of taurine to fit into the β-adrenergic receptor, we suspect taurine might also interact with other adrenergic binding sites involved in neuronal reuptake of norepinephrine or the regulation of reuptake. These considerations raise the interesting possibility that taurine in the extracellular space might act to modulate transynaptic contol of pineal function by interacting with either pre- or postsynaptic adrenergic binding sites.

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