Studies on Adenosine 3′,5′-Phosphate in Rabbit Cerebral Cortex

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SUMMARY

Incubation of slices of rabbit cerebral cortex with histamine resulted in large accumulations of adenosine 3′,5′-phosphate. Maximal levels of the nucleotide required about 15 min exposure to histamine and represented more than an 8-fold increase. Inclusion of theophylline (0.5 mM) in incubation media had little effect by itself on the accumulation of adenosine 3′,5′-phosphate but increased the effect of histamine 3-fold. Norepinephrine increased the nucleotide level only about 70%, but only 2 min exposure to the agent was required to produce maximal values. This pattern of responses is in sharp contrast to that observed in cerebellar slices, but appears to be similar to that of other areas of rabbit brain. The content of adenosine 3′,5′-phosphate in the cerebral cortex of rabbit heads frozen rapidly at intervals after decapitation rose about 8-fold within 90 sec after decapitation. Although phosphorylase *a* levels increased very rapidly after decapitation, no changes in phosphorylase *a* content of either cerebellar or cerebral cortex slices was detected under conditions producing large increases in adenosine 3′,5′-phosphate.

INTRODUCTION

Investigation of the functional role of cyclic 3′,5′-AMP[2] in the central nervous system in this laboratory began with a study of the factors influencing the accumulation of the nucleotide in isolated cerebellar tissue (1, 2). It was observed that in the presence of NE, the level of cyclic 3′,5′-AMP increased more than 6-fold within 6 min and fell rather rapidly with longer incubations. Histamine was found to produce similar but smaller effects, while serotonin produced only 2- to 3-fold increases.

This report is concerned in part with some parallel observations using slices of other anatomical areas of rabbit brain, primarily cerebral cortex. Incubation of slices of cerebral cortex with histamine resulted in large increases in cyclic 3′,5′-AMP accumulation which developed relatively slowly and were well sustained. On the other hand, NE produced only small changes in cyclic 3′,5′-AMP levels which developed more rapidly. With the use of slices from both cerebral cortex and cerebellum, large increases in cyclic 3′,5′-AMP were not accompanied by appreciable changes in the content of phosphorylase *a*. Finally, the cyclic 3′,5′-AMP content of cerebral cortex tissue was found to increase nearly 10-fold within 90 sec after decapitation of an animal, while phosphorylase *a* levels reached a maximum even more rapidly.

MATERIALS AND METHODS

The procedures for the sacrifice of the rabbits, the preparation and incubation of slices, the determination of cyclic 3′,5′-AMP and the estimation of protein were the same as those described in the preceding report (2). For experiments studying events immediately following decapitation, the rabbits were decapitated with a special guillo-

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[2] The abbreviations used are: cyclic 3′,5′-AMP, adenosine 3′,5′-phosphate; NE, L-norepinephrine.

tine, the scalps were cut down to the bone with a single stroke of a sharp scalpel, the cut edges were pulled apart and the entire head was plunged into 2 liters of isopentane which had been chilled in liquid N_2 until all the inner surfaces of the container were coated with crystals. The minimum time required between decapitation and immersion into isopentane was about 5 sec. The isopentane was vigorously stirred about the head for 6 min before the head was transferred to solid CO_2 for storage. The heads were split in half and while immersed in liquid N_2, samples of cerebral cortex lying immediately beneath the center of the skull were scooped out and were stored at —65° until used.

Phosphorylase was determined on aliquots of frozen tissue by homogenizing rapidly 35–140 mg in 7 ml of an ice cold solution which contained 75 mM NaF and 5 mM EDTA (adjusted to pH 6.7). The amount of tissue was estimated by eye and was calculated from a subsequent determination of protein (2). Aliquots of 0.25 and 0.5 ml of homogenates were incubated in a system with a final volume of 2.0 ml containing at final concentration 11.2 mM glucose 1-phosphate (adjusted to pH 6.1), 0.1 M NaF, 3.4 mM EDTA, and 1.25 mg of glycogen per milliliter; other aliquots were incubated in mixtures which included 2.5 mM 5′-AMP or which contained no glycogen. The incubations were terminated by the addition of 2 ml of cold 3% trichloroacetic acid. The inorganic phosphate present in an equivalent of 0.5 ml of incubation mixture was determined by the method of Fiske and SubbaRow (3) as adapted to the Klett-Summerson photometer. The difference between the inorganic phosphate produced in the presence and in the absence of glycogen was considered due to phosphorylase action. The rate of inorganic phosphate formation was linear with time up to 80 min of incubation and proportional to the amount of homogenate used as long as the total did not exceed 15% of the glucose 1-phosphate originally present. In the presence of 5′-AMP, approximately 3.5 μmoles of inorganic phosphate were produced per milligram of protein in 60 min, which is equivalent to about 8.7 μmoles per minute per gram wet weight. The ratio of activity observed in the absence of 5′-AMP to that in its presence was used as an index of the relative amount of phosphorylase *a* present in a given sample; this ratio was observed to range from a minimum of 0.11 to a maximum of 0.80. This ratio was not observed to be related to the amount of tissue assayed within the limits stated above; this was taken as evidence that endogenous 5′-AMP was not appreciably influencing the estimation of the relative amount of phosphorylase *a*. In addition, it can be calculated that the assay of 0.5 ml of the most concentrated homogenate used (2%) would result in a final concentration of about 18 μM 5′-AMP if all the adenine nucleotides were present in the form of 5′-AMP and if the concentration of total adenine nucleotide in rabbit brain were the same as observed by Lowry *et al.* in mouse forebrain (4). Thus, less than 10% of the phosphorylase *b* activity could be included in the estimation of phosphorylase *a* activity, using the value of 0.11 mM as the apparent K_m of brain phosphorylase *b* for 5′-AMP (5).

RESULTS

In preliminary experiments, the effects of NE and histamine on the level of cyclic 3′,5′-AMP in slices derived from various areas of rabbit brain were compared. Two such experiments are summarized in Table 1. In all four tissues tested, histamine increased cyclic 3′,5′-AMP levels at least 8-fold. On the other hand, NE produced large effects only in cerebellar tissue. In a similar experiment, exposure of slices of caudate nucleus to histamine for 6 min increased cyclic 3′,5′-AMP levels more than 7-fold whereas NE produced less than a 50% increase. In another experiment, in which a mixture of slices from the brachium pontis, fornix, and optic tract were used, exposure of the tissue to either histamine or NE for 10 min resulted in about a 3-fold increase in cyclic 3′,5′-AMP. It is possible that at least one of these three tissues will be shown to resemble the cerebellum in its response to NE.

TABLE 1

Cyclic 3',5'-AMP accumulation in slices from various areas of rabbit brain

Each experiment was performed with tissue pooled from 3 animals. In experiment II, the animals had been injected subcutaneously with reserpine (2.5 mg/kg) 24 hr before sacrifice. All incubations were carried out in the presence of 0.5 mM theophylline and consisted of 42 min of preincubation followed by 10 min exposure to the hormones at 0.1 mM.

Tissue	Cyclic 3',5'-AMP content (pmoles/mg protein)			
	Control	NE	Histamine	NE + Histamine
Experiment I				
Cerebral cortex	3.0	5.3	49	68
Cerebellum	3.5	50	35	105
Hypothalamus	4.7	8.7	99	160
Experiment II				
Cerebral cortex	7.9	11.2	94	101
Cerebellum	7.2	82	63	170
Hypothalamus	10.0	17.3	310	500
Reticular formation (brain stem)	8.2	18.2	280	260

The experiments described above provide only a rough comparison of the response to these agents in different anatomical areas of rabbit brain; it is quite possible that important characteristics of the response are not revealed by making observations with a single concentration at a single point in time. For example, values for cerebellar tissue would be appreciably less after 10 min than after 6 min of exposure to either agent (2). On the other hand, 10 min may not be long enough for histamine to exert its full effect in cerebral cortical slices (Fig. 1). So far only the characteristics of the response in cerebral cortical and cerebellar tissue (2) have been examined in any detail.

Influence of NE on Cyclic 3',5'-AMP in Cerebral Cortical Slices

While the effects of NE on the level of cyclic 3',5'-AMP in slices of cerebral cortex were comparatively small, these effects were consistently observed. In 12 experiments in which slices of cerebral cortex had been exposed to NE at 50 μM or higher for 2 min or longer, the content of the cyclic nucleotide rose $68 \pm 5\%$ (SEM) yielding a p value of less than 0.001. The effects of NE were maximal within 2 min and tended to decline upon longer exposures. In 3 experiments, the level of cyclic 3',5'-AMP after 2 min of exposure to NE was found to be higher by 1.07 ± 0.07 pmoles per milligram of protein than after 6 min of exposure to the agent ($p < 0.005$). In one experiment exposure to NE for 45

TABLE 2

Cyclic 3',5'-AMP level in slices of cerebral cortex as a function of concentration of histamine and norepinephrine

Tissue from 6 animals was pooled. Incubation was carried out in 0.5 mM theophylline. After 42 min of preincubation, slices were exposed to various amounts of NE or histamine for 6 and 20 min, respectively.

Hormone	Concentration (μM)	Cyclic 3',5'-AMP[a] (pmoles/mg protein)
NE	0.5	4.6
NE	5.0	5.0
NE	50.0	5.9[b]
Histamine	0.1	6.0
Histamine	1.0	9.5
Histamine	10.0	60
Histamine	100.0	132[c]

[a] The values for slices incubated without hormones for 6 and 20 min were 3.3 and 3.5 pmoles per milligram of protein, respectively.

[b] The values for slices incubated with 50 μM NE for 2 and 20 min were 6.9 and 5.7 pmoles per milligram of protein, respectively.

[c] The values for slices incubated with 100 μM histamine for 6 and 40 min were 74 and 23 pmoles per milligram of protein, respectively.

and 75 sec yielded values no higher than that after 2 min exposure.

Not enough data have been accumulated to define adequately the sensitivity of cerebral cortical slices to NE. The experiment summarized in Table 2 does suggest that

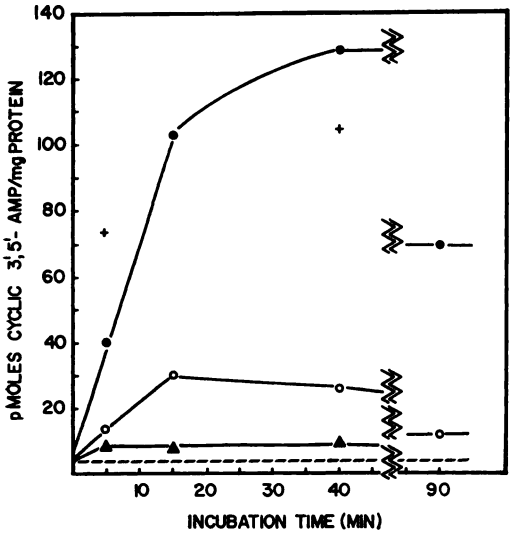

FIG. 1. *Time course of cyclic 3',5'-AMP content of cerebral cortex slices after addition of NE or histamine*

Slices were incubated for 42 min prior to addition of sufficient NE or histamine to achieve a final concentration of 0.1 mM. The middle curve (○—○) represents values observed after addition of histamine in the absence of theophylline. The remaining curves represent values observed in the presence of 0.5 mM theophylline after the addition of NE (▲—▲) or histamine (●—●). Two values observed after the combined addition of NE and histamine in the presence of theophylline are also represented (+). The values observed for slices incubated a total of 57 min in the absence of the amines in the presence and absence of 0.5 mM theophylline were 4.5 and 3.7 pmoles/mg protein, respectively (represented by dashed line).

the sensitivity is no less than that for cerebellar tissue; in the latter tissue, half-maximal effects were observed at about 4 μM NE (2). Another experiment, in which cyclic 3',5'-AMP was increased 47% at 2 μM NE and 80% at 50 μM NE also supports this suggestion.

Influence of Histamine on Cyclic 3',5'-AMP in Cerebral Cortical Slices

In most experiments, 0.5 mM theophylline was included in the incubation media. It appeared that theophylline produced similar effects in slices of both cerebral cortex and cerebellum: theophylline by itself increased the levels of cyclic 3',5'-AMP only slightly, but magnified the effects of histamine about 3-fold (Fig. 1). While the time course of the effects of histamine on cerebral cortical slices was somewhat unpredictable, the contrast with time courses observed in cerebellar slices is obvious. In the experiment depicted in Fig. 1, it appeared that at least 15 min of exposure to histamine was required to reach maximal levels and that thereafter the rate of disappearance of the nucleotide was relatively slow. In other experiments, it appeared either that maximal levels were achieved more rapidly (Table 3) or that the nucleotide disappeared more rapidly (Table 2) than would be expected from the data in Fig. 1. However, it seemed safe to assume that, in any given experiment, the values observed after 20 min exposure to histamine would be approximately maximal; with this exposure time, 10 μM histamine produced approximately half-maximal effects (Table 2). In this regard cerebral cortical slices were similar to cerebellar slices which responded half-maximally to 10 μM histamine in 6 min, an exposure time producing maximal values (2).

In other experiments, the effects of 20 min exposure to 10 μM histamine were reduced by more than 60% in the presence of 5 μM diphenhydramine and by more than 80% in the presence of 50 μM chlorpromazine; these results are also similar to those obtained with cerebellar slices (2). Experiments with adrenergic blocking agents have not been attempted as yet. Because of the large difference in the size of the individual responses to NE and histamine, it is not possible to decide whether the combined addition of the agents produced additive effects as was the case with cerebellar slices. On the other hand, in only two of eleven observations were the values ob-

TABLE 3

Effect of various agents on cyclic 3',5'-AMP content of slices of cerebral cortex

Tissue from five animals was pooled. All incubations were in the presence of 0.5 mM theophylline and consisted of 42 min of preincubation followed by exposure to the agents at 0.1 mM for the periods indicated.

Agent	Cyclic 3',5'-AMP content[a] after an incubation period of			
	2 min	6 min	20 min	40 min
None	—	3.8	4.0	4.4
NE	—	6.7	7.0	5.2
Histamine	—	90	102	90
NE + histamine	—	73	104	132
Serotonin	4.8	3.8	4.1	—
Dopamine	4.2	4 5	5.2	—

[a] Picomoles per milligram of protein.

served in the presence of both agents lower than the corresponding values for histamine alone.

Effects of Other Agents

In the experiment summarized in Table 3 dopamine and serotonin were observed to produce values for cyclic 3',5'-AMP within 30% of controls; similar results were seen in other experiments. It seems clear that exposure to serotonin for 6 min or longer or to dopamine for any length of time failed to change cyclic 3',5'-AMP levels significantly. However, it is not yet clear whether brief exposures to serotonin were without significant effect. In four experiments, exposure to serotonin for 2 min produced increases averaging 51% ± 21% (SEM; range 15–105%), yielding a p value between 0.05 and 0.1.

In other experiments, incubation of slices in the presence of acetylcholine (10 mM), glutamate (4 mM), and γ-aminobutyrate (4 mM) for varying times failed to change cyclic 3',5'-AMP levels by more than 30%. In one experiment incubation of slices with 100 mM KCl in the presence of 0.5 mM theophylline increased the content of cyclic nucleotide by no more than 50%.

Lack of Relationship between Cyclic 3',5'-AMP and Phosphorylase a Content of Brain Slices

In preliminary experiments in which slices of cerebellum were used, phosphorylase activity measured in the absence of 5'-AMP was observed to be between 16% and 21% that measured in the presence of 5'-AMP regardless of the level of cyclic 3',5'-AMP found. These experiments involved prior incubation of the slices for 42 min followed by 6 min of exposure to various amounts of NE or blank solutions. In the experiment summarized in Table 4, the time of incubation before exposure of slices to NE or histamine was varied from 0 to 80 min. It can be seen that the phosphorylase a content of slices fell rapidly upon incubation at 37°, reaching a minimum within 26 min and that the addition of NE or histamine or a combination of the two had little effect. In addition, aliquots of slices incubated for 26 and 56 min were homogenized and incubated for 30 min in the presence of 2.4×10^{-3} M ATP, 8×10^{-3} M $MgSO_4$ and 2.2×10^{-2} M NaF. After removal of adenine nucleotides by treatment with Dowex-1 (fluoride form), the phosphorylase activity of the incubation mixtures measured in the absence of 5'-AMP was found to have increased to between 63% and 81% of that measured in the presence of 5'-AMP. These results indicate that the phosphorylase in incubated brain slices was in a form that could be converted to phosphorylase a by the endogenous phosphorylase b kinase, rather than in a partially degraded form analagous to the species produced by treating phosphorylase a from skeletal or cardiac muscle with trypsin (6, 7).

A similar lack of correlation between

TABLE 4

Phosphorylase a content of cerebellar slices incubated with norepinephrine and histamine

The tissue used was obtained from 6 rabbits, pooled after slicing. After incubation at 37° for the indicated times, sufficient NE or histamine or a combination of the two was added to achieve a final concentration of 0.1 mM. At 6 min after the addition of hormone, the slices were frozen in liquid N_2. A portion of each frozen slice was homogenized and assayed for phosphorylase activity.

Incubation time before hormone addition	Hormone added	Phosphorylase activity without 5'-AMP (%)
None	None	27[a]
None	NE	28
None	Histamine	26
None	NE + Histamine	31
20 min	None	19
20 min	NE	18
20 min	Histamine	18
20 min	NE + Histamine	19
50 min	None	19
50 min	NE	19
50 min	Histamine	23
50 min	NE + Histamine	21

[a] The value observed in slices which had not been incubated at 37° was 70%.

3',5'-AMP and phosphorylase *a* levels was observed in experiments in which slices of cerebral cortex were used. The experiment summarized in Table 5 shows that phosphorylase *a* activity declined rapidly as soon as the incubation of slices commenced even though the concentration of cyclic 3',5'-AMP actually rose about 50% during the first 1.5 min of incubation. Furthermore, the addition of histamine 8 min after initiation of incubation produced an increase in cyclic 3',5'-AMP, while the phosphorylase *a* activity continued to decline. Although the decline in phosphorylase *a* appeared to be slower in the presence of histamine in this experiment, in other experiments histamine had no effect. On the other hand, the increase in cyclic 3',5'-AMP during the first few minutes of warm incubation was a consistent finding provided that 80 sec or more had elapsed between decapitation of the animal and immersion of the brain tissue in ice-cold medium. No satisfactory explanation of these observations has been found.

These results in brain slices appear to contrast sharply with the correlation between increased levels of cyclic 3',5'-AMP and phosphorylase activity observed in prefused rat hearts (8), in rat skeletal muscle *in situ* (9), and in slices of rabbit liver (Table 6) upon exposure of the tissue to epinephrine. In the experiment summarized in Table 6, the level of cyclic 3',5'-AMP increased more than 2-fold within 3 min after the addition of epinephrine, whereas the phosphorylase activity increased about 80%. However, the maximum phosphorylase activity achieved in the presence of epinephrine was less than 40% of that

TABLE 5

Phosphorylase a and cyclic 3',5'-AMP content of slices of cerebral cortex

The tissue from 3 animals was dissected in the cold. After removal and freezing of blocks of the cerebral cortex from each animal, the remainder was sliced and pooled. Aliquots were incubated at 37° and frozen as indicated. The frozen tissue was powdered and divided into two portions, one extracted with HCl and assayed for cyclic 3',5'-AMP and the other homogenized and assayed for phosphorylase activity.

Treatment	Phosphorylase activity without 5'-AMP (%)	Cyclic 3',5'-AMP (pmoles/mg protein)
Blocks fixed 5 min after decapitation	80	56
Slices fixed just before incubation	62	26
Slices incubated 1½ min	44	38
Slices incubated 3 min	32	31
Slices incubated 8 min	23	28
Slices incubated 20 min	11	18
Slices incubated 8 min without and 12 min with 1×10^{-4} M histamine	16	39

found in liver slices prior to incubation. Thus it is possible that the lack of correlation between increases in cyclic 3′,5′-AMP and phosphorylase *a* in brain slices was due to the slicing and incubation procedures and does not necessarily indicate that cyclic 3′,5′-AMP plays no part in the regulation of phosphorylase activity in brain tissue.

TABLE 6

Phosphorylase activity and cyclic 3′,5′-AMP content of rabbit liver slices

The liver was removed from a decapitated, bled rabbit and was chilled in cold medium. One lobe was dissected into blocks which were sliced using the McIlwain tissue chopper. The slices were dispersed and distributed into baskets in portions of 150–200 mg each. Each portion was shaken at 37° in air in 30 ml of medium containing at final concentration 120 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 2.6 mM $CaCl_2$, 15.6 mM sodium phosphate (pH 7.35), 0.02 mM sodium EDTA, and 2 mg of glucose per milliliter. When present the concentration of L-epinephrine was 0.05 mM and histamine was 0.1 mM. The slices were transferred to fresh medium after 30 min; after 16½ to 30 min of additional incubation, each portion of slices was rinsed briefly in 30 ml of 164 mM NaCl and frozen in liquid N_2. A portion of each sample was homogenized rapidly in an ice-cold solution containing 75 mM NaF and 5 mM EDTA (pH 6.7) and assayed for phosphorylase activity in the presence of 5′-AMP. The remainder was used for the determination of cyclic 3′,5′-AMP.

Incubation time (min)	Phosphorylase (μmoles P_i/hr/mg protein)	Cyclic 3′,5′-AMP (pmoles/mg protein)
None	2.62	3.3
15	0.52	—
30	0.46	—
51	0.51	2.0
45 + 1½ min with epinephrine	0.65	3.7
45 + 3 min with epinephrine	0.92	5.3
45 + 6 min with epinephrine	0.87	5.1
45 + 15 min with epinephrine	1.01	3.5
45 + 3 min with histamine	0.51	1.9

Levels of cyclic 3′,5′-AMP and phosphorylase a immediately after decapitation

It was noted that cerebral tissue analyzed prior to incubation contained relatively large amounts of cyclic 3′,5′-AMP (Table 5), even more than that found after incubation of slices with histamine in the absence of theophylline (Fig. 1). The content of cyclic 3′,5′-AMP in the cerebral cortex of rabbits whose heads were frozen at various times after decapitation was then determined. These results are summarized in Table 7. The level of the cyclic nucleotide increased on the average about 8-fold in the interval between 5 and 90 sec after decapitation. Breckenridge reported that the levels of cyclic 3′,5′-AMP in mouse cerebral cortex taken from heads frozen 20 sec after decapitation were about twice that of tissue frozen *in situ* (10). Also included in Table 7 are the results obtained using animals decapitated under barbiturate anesthesia. The increase in cyclic 3′,5′-AMP was slowed and attenuated significantly. In both series the lowest level (8.2 pmoles per milligram of protein) was observed in tissue taken from a head frozen 5 sec after decapitation of an anesthetized animal. This corresponds to about 1.1 μmole per kilogram of tissue, compared to the value of 0.9 μmole per kilogram of tissue reported by Breckenridge (10) for cortical tissue of anesthetized mice frozen *in situ*.

Pretreatment of animals with either reserpine or the antihistaminic compound diphenhydramine apparently had little influence on the level of cyclic 3′,5′-AMP after decapitation (Table 7). However, it is possible that significant differences would emerge from a larger number of observations. It seems more likely that a greater number of experiments using chlorpromazine would reveal a significant effect on the rate of accumulation of cyclic 3′,5′-AMP after decapitation. Any future experiments should include provisions for control of body temperature in order to eliminate drug- induced hypothermia as a significant factor.

In some instances aliquots of frozen

TABLE 7

Cyclic 3',5'-AMP concentration in rabbit cerebral cortex frozen after decapitation

Rabbit heads were plunged into isopentane chilled in liquid N_2 at the indicated time after decapitation and were processed as described under Methods. Animals treated with pentobarbital received 30 mg/kg intravenously (ear vein) about 10 min prior to sacrifice. Animals treated with reserpine received three successive subcutaneous injections of 0.5 mg/kg, 1.0 mg/kg, and 2.5 mg/kg 68 hr, 44 hr, and 20 hr, respectively, before sacrifice. Animals treated with diphenhydramine or chlorpromazine were injected intraperitoneally with 15 mg/kg about 80 min prior to sacrifice. Results are expressed as picomoles of cyclic 3',5'-AMP per milligram of protein ± standard error of the mean.

Time after decapitation	Drug injected				
	None	Pentobarbital	Reserpine	Diphenhydramine	Chlorpromazine
5 sec	13.3 ± 0.88 (3)[a]	8.3 (1)	13.5 ± 0.1 (2)	—	—
30 sec	34.7 ± 1.9 (4)	14.4 ± 3.2[b] (3)	26.5 (1)	24.0 (1)	18.0 (1)
60 sec	49.3 ± 6.7 (3)	—	42.8 ± 2.5 (2)	—	—
90 sec	88.0 ± 12.3 (5)	42.6 ± 2.6[c] (3)	77.0 (1)	74.0(1)	48.0 (1)
3 min	—	—	93.0 (1)	—	—
4 min	77.5 ± 13.6 (4)	46.0 ± 29.0 (2)	—	102.0 (1)	20.5 (1)

[a] Number of animals given in parentheses.
[b] Significantly different from control animals ($p < 0.005$).
[c] Significantly different from control animals ($p < 0.05$).

cerebral cortex were assayed for phosphorylase *a* activity as well as for cyclic 3',5'-AMP. The results of two experimental series, one with and one without pentobarbital anesthesia, are presented in Table 8. In unanesthetized animals, the phosphorylase *a* content of cerebral tissue appeared to have reached nearly a maximal value in the heads frozen as rapidly as possible after decapitation (i.e., immersion in chilled isopentane within 5 sec). Although the corresponding value in the anesthetized series was lower, the phosphorylase *a* activity was already probably half-maximal. Despite the use of a different species and a different freezing technique, these results resemble those of Breckenridge and Norman (5). These workers reported that the phosphorylase activty without 5'-AMP in whole mouse brain rose from about 20% (frozen by pouring Freon-12 chilled in liquid N_2 onto the exposed skull) to about

TABLE 8

Phosphorylase a and cyclic 3',5'-AMP content of rabbit cerebral cortex frozen after decapitation

Rabbit heads were plunged into isopentane chilled in liquid N_2 at the indicated time after decapitation. Aliquots of cerebral cortex tissue were assayed either for cyclic 3',5'-AMP or for phosphorylase activity. Animals treated with pentobarbital received 30 mg/kg intravenously about 10 min before sacrifice.

Time after decapitation (sec)	Unanesthetized		Anesthetized with pentobarbital	
	Cyclic 3',5'-AMP (pmoles/mg protein)	Phosphorylase activity without 5'-AMP (%)	Cyclic 3',5'-AMP (pmoles/mg protein)	Phosphorylase activity without 5'-AMP (%)
5	12.8	64	8.3	40
30	37	67	8.7	50
60	61	74	27	75
90	138	76	45	71
120	95	64	39	72

69% immediately after decapitation (frozen by immersion into liquid N_2); the latter value was significantly reduced when anesthetized animals were used. If it is presumed that levels of phosphorylase *a* and cyclic 3′,5′-AMP in rabbit cerebral cortex prior to decapitation were the same as those reported for mouse cerebral cortex, then the data in Table 8 would indicate that a relatively small increase in cyclic 3′,5′-AMP content (50–60%) was associated with a large increase in phosphorylase *a*. This tentative conclusion would be best verified using small animals and solvents chilled in liquid N_2 for freezing to achieve the fastest possible rate of fixation.

DISCUSSION

The complex patterns of effects of NE and histamine on the accumulation of cyclic 3′,5′-AMP in slices of cerebral cortex and cerebellum are very difficult to interpret and probably reflect the degree of cellular heterogeneity of central nervous system tissue. It is tempting to consider the possibility that the time course of the effect of NE in slices of cerebral cortex is an accelerated version of that in cerebellar tissue. This hypothesis would hold that the level of cyclic 3′,5′-AMP in each responding unit would rise and fall at rates exceeding the rate of penetration of NE into the vicinity of the responding unit and that only the later and smaller plateau responses of individual units would summate to any appreciable degree. While there is little evidence at present to support such a view, it may become important to evaluate this possibility in order to decide either whether a given agent should be considered "inactive" or how changes in the metabolism of cyclic 3′,5′-AMP may be related to functional alterations of central nervous system structures.

The observations presented here do not provide a basis for understanding how decapitation induces a rapid accumulation of of cyclic 3′5′-AMP in the cerebral cortex. It is unlikely that the release of NE could be responsible for an appreciable portion of this phenomenon considering both the magnitude of the maximal effect of NE upon cerebral cortex slices and the apparent lack of effect of prior treatment with reserpine upon the events subsequent to decapitation. While it is more difficult to eliminate a possible major role for the release and action of histamine, there is little evidence supporting such a proposition. Any hypothesis centering on histamine would require (a) mechanisms for achieving concentrations of histamine approaching 100 μM in the vicinity of the histamine-reactive sites when the average concentration in cerebral tissues is probably less than 1 μM (b) an explanation of the failure to observe a prominent effect of diphenhydramine upon the accumulation of cyclic 3′,5′-AMP after decapitation, and (c) an explanation of the much slower rate of accumulation of the cyclic nucleotide in cerebral cortex slices exposed to histamine relative to that observed after decapitation. The first of the preceding explanations could be provided within the framework of the concept of synaptic units. If, as recent evidence suggests (11), the histamine of the cerebral cortex is contained predominantly within small nerve endings and if the histamine-reactive sites are confined primarily to adjacent subsynaptic regions, then achievement of sufficient local concentrations can be easily visualized. In addition, difficulty in achieving blockade by pharmacologic antagonists might also be expected by analogy with the behavior of many synaptic junctions in the peripheral autonomic nervous system. Finally, the synaptic concept might assist in reconciling the apparent discrepancy in rates even though it would also be necessary to propose either a very slow rate of penetration of histamine into slices or a comparatively slow change in the rate of synthesis of cyclic 3′,5′-AMP after histamine reaches an effective concentration.

One alternative explanation for the rise in cerebral cyclic 3′,5′-AMP after decapitation would involve the release and action of unidentified endogenous substances, perhaps including compounds belonging to the category of neurohormones. A second alternative would ascribe changes in cyclic 3′,5′-AMP to the oxygen and glucose deprivation

following decapitation. However, in preliminary experiments incubation of slices of guinea pig cerebral cortex in the absence of either glucose or oxygen failed to produce detectable increases in the cyclic nucleotide.[3] These two alternatives are not mutually exclusive, and the interruption of circulation following decapitation could allow the accumulation of metabolites and/or neurohumoral substances released as a consequence of anoxia.

As yet, changes in levels of cyclic 3′,5′-AMP have not been correlated with any metabolic changes in slices of brain tissue. In addition to the failure to observe increases in phosphorylase *a* levels in brain slices reported here, other experiments have indicated that histamine does not induce changes in lactate output by slices of guinea pig cerebral cortex.[3] Although variation of a number of experimental conditions has not altered these negative findings, it is possible that some aspect of the *in vitro* experimental procedures has prevented observing one or more consequences of the elevation of cyclic 3′,5′-AMP content of brain slices. On the other hand, it is possible that cyclic 3′,5′-AMP has no part in the regulation of phosphorylase *a* levels in brain tissue. This would leave the rapid changes in brain phosphorylase *a* observed after decapitation (5) or after N_2-breathing (12) essentially unexplained.

[3] A. Sattin and T. W. Rall, unpublished observations.

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