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Cyclic AMP in the Rat Brain: Microwave Irradiation as a Means of Tissue Fixation

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I. INTRODUCTION

Cyclic AMP levels are subject to rapid fluctuations in most tissues. The method of fixation is therefore an important consideration in any study dealing with the effects of drugs and hormones upon these levels. Perhaps the most generally useful procedure has been to clamp the tissue between two blocks of aluminum at the temperature of liquid nitrogen (Wollenberger, Ristau, and Schoffa, 1960), causing almost instantaneous freezing of deep as well as surface cells. The frozen tissue is then pulverized at the temperature of liquid nitrogen, and the frozen powder rapidly homogenized in the presence of a protein denaturant. Interfering enzymes are thus thawed and denatured more or less simultaneously. The supernatant can then be purified and assayed for its cyclic AMP content, and this will presumably reflect the amount of cyclic AMP present in the tissue at the instant it was frozen.

Although this technique has been useful in the study of many systems, such as the perfused rat heart (see Robison, Butcher, Øye, Morgan, and Sutherland, 1965), it is obviously impractical for the study of organs such as the kidney or brain, in which discrete groups of cells serve different functions and respond differently to drugs and hormones.

Even the heart is far from homogeneous, of course, but at least it was possible in that case to assume that *most* of the tissue mass was composed of myocardial cells, and that the overall level of cyclic AMP would primarily

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reflect events occuring in those cells. By contrast, whole-brain measurements could not be assumed to reflect changes occurring in a particular area, such as the hypothalamus, for example.

One possible method for studying cyclic AMP changes in different areas of the rat brain *in situ* might be to freeze the brain by immersing whole rats in liquid nitrogen (or in isopentane at the temperature of liquid nitrogen), followed by dissection of the frozen tissue. However, there are two major drawbacks to this procedure. First, although the cortex might be frozen quite rapidly, inactivation of deeper structures would be far from instantaneous (Richter and Dawson, 1948; Takahashi and Aprison, 1964; Jongkind and Bruntink, 1970). In an adult rat, for example, it may take as long as 70 sec for the thalamus to fall from body temperature to O°C, and even at this temperature, as Goldberg and his colleagues have shown (O'Dea, Haddox, and Goldberg, 1971), phosphodiesterase retains considerable activity. Thus catabolism of cyclic AMP would continue in lower brain areas, leading to artifactually low levels of the nucleotide in these areas. The other main objection to this technique, even if the values so obtained were valid, is that it is extremely difficult and time-consuming to dissect a frozen brain accurately.

In view of these difficulties, we have explored the possibility of using microwave irradiation as a means of tissue fixation for studying cyclic AMP levels in the rat brain *in situ*. The preliminary results summarized in this paper have led us to believe that this is a promising technique for this purpose. Throughout these studies we have been following the lead of Stavinoha, Pepelko, and Smith (1970), who had earlier applied the microwave technique to the study of brain acetylcholine levels.

II. EXPERIMENTAL

The source of microwave irradiation (MI) used in these studies was a commercial oven (Litton Model 550) with a power output of 1,250 watts and a frequency of 2,450 mc. Its use for this purpose has been described elsewhere (Schmidt, Speth, Welsch, and Schmidt, 1972*a*). In summary, the rat is oriented by placing it in front of a styrofoam tunnel which the rat readily enters without coaxing or trauma. The styrofoam tunnel, which does not absorb MI, is placed in the center of the unit facing the power source. Although the power of this unit is very small compared to that which could be built into an experimental model, and the radiation diffuse and unfocused, the rat is dead within 5 sec after the apparatus is activated. In some cases death is preceded by a brief convulsion. When mice are used death appears to be instantaneous.

Brain temperature was determined after varying periods of MI by means of a micro thermocouple inserted into the thalamic area of the brain. Temperature as a function of duration of MI is plotted in Fig. 1. It can be seen that in

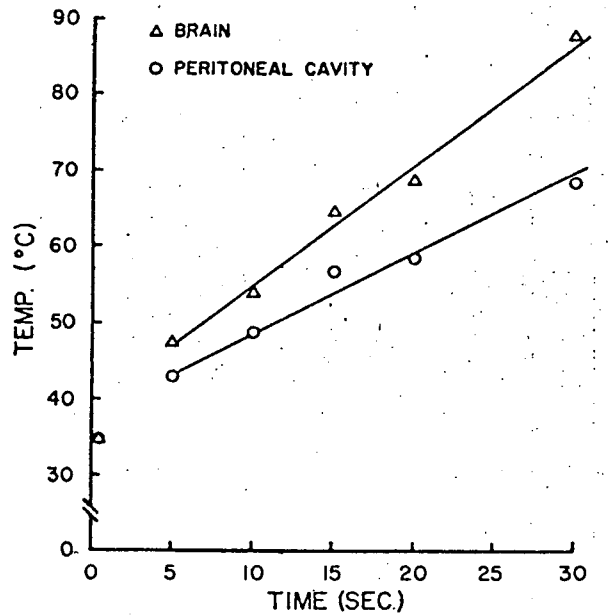

FIG. 1. Effect of MI on thalamic and peritoneal temperatures of the rat. (From Schmidt et al. 1972*a*).

the brain a temperature of 70°C is reached within 20 sec; peritoneal temperatu rises more slowly, being approximately 55°C at this time.

It was presumed that enzymes would be inactivated by MI as a resu of heat denaturation, so that this technique can only be applied to the measur ment of heat-stable compounds. The heat stability of cyclic AMP had be recognized ever since its discovery, and we established that aqueous solutio of cyclic AMP could be exposed to MI without change. The MI techniq has also been used to study acetylcholine in the brain (Schmidt, Schmidt, a Robison, 1971), but will not be applicable for the study of many other co pounds, especially proteins. Pituitary growth hormone, for example, w almost absent after 5 sec of MI and was completely obliterated after 15 sec exposure (G. Cehovic, *unpublished observations*).

In initial experiments we measured cyclic AMP levels in the whole bra after varying periods of MI. Rats were decapitated immediately after MI a the heads packed in ice. The brains were then removed and cut in half. On half of each brain was homogenized in 0.1 N HCl exactly 5 min after exposu to MI. The other half was maintained at ice-bath temperature for an addition 30 min before being homogenized. As seen in Table 1, 10 sec of MI was insu ficient for complete tissue fixation. The level of cyclic AMP in the brain ha homogenized 35 min following MI was more than twofold higher than th

TABLE 1. *Effect of duration of microwave irradiation on cyclic AMP levels in whole brains*[a]

Duration of exposure to MI	Cyclic AMP (nmoles/g)	
	Homogenized 5 min after MI	Homogenized 35 min after MI
10 sec	0.50	1.28
20 sec	0.81	0.79
30 sec	0.92	0.87

[a]Brains were placed on ice immediately after MI. One-half of each brain was homogenized in 0.1 N HCl 5 min later, while the remaining half rested on a cooled porcelain plate for an additional 30 min before homogenization. Each value represents the mean of two to four separate determinations. (From Schmidt et al., 1972*b*.)

prepared only 5 min after MI. Apparently, the surviving adenylyl cyclase activity predominates over phosphodiesterase in the brain under these conditions. After 20 sec of MI, however, the level of cyclic AMP remained constant regardless of post-MI homogenization time suggesting that 20 sec was sufficient to inactivate both enzymes. This was confirmed by measuring the activities of adenylyl cyclase and phosphodiesterase in homogenates of brains from rats exposed to varying periods of MI (Fig. 2). As a result of these studies, 30 sec was chosen as the exposure time for subsequent experiments.

A dramatic increase in the brain level of cyclic AMP occurs following decapitation (Breckenridge, 1964; Kakiuchi and Rall, 1968). Previous studies had examined this effect only in the whole brain. We were now in a position to investigate this phenomenon further to determine the contribution made by specific areas to the overall fivefold increase seen in the whole brain. We first established that cyclic AMP was not evenly distributed throughout the brain of rats killed by MI. Levels were highest in the cerebellum and brainstem, intermediate in the hypothalamus and midbrain, and lowest in the hippocampus and cortex (Schmidt et al., 1971), correlating roughly with the *ratio* of adenylyl cyclase to phosphodiesterase as measured in homogenates of these areas (Weiss and Costa, 1968). When rats were decapitated first and then exposed to MI at various times after the decapitation, it was found that although cyclic AMP increased in all areas, the cerebellum contributed far more to the overall rise than did any of the other areas (Fig. 3). This serves to illustrate the danger of using decapitation as the method of sacrifice in pharmacological studies dealing with cyclic AMP in the brain. A substantial drug-induced change in a small area could be easily overshadowed by the massive decapitation-induced increase occurring elsewhere.

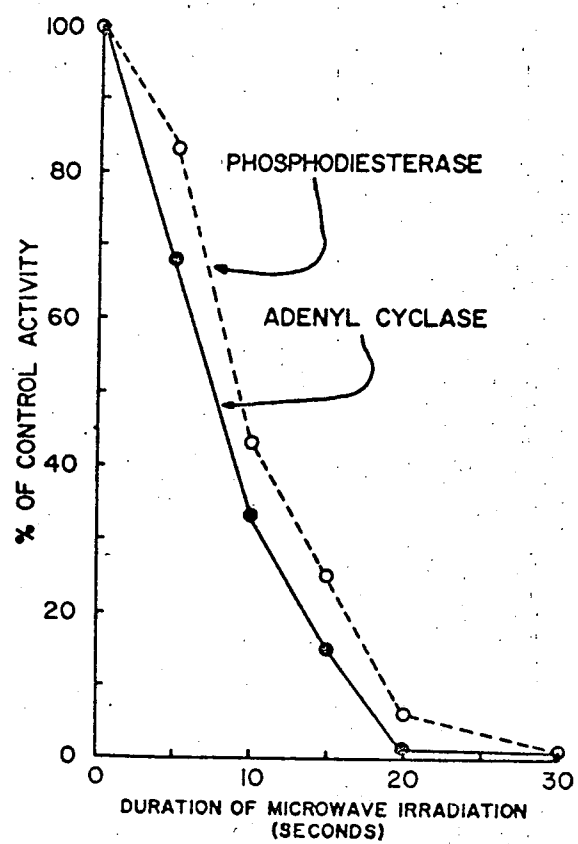

FIG. 2. Effect of MI on cyclase and phosphodiesterase activities in rat brain homogenates. Rats were exposed to MI for the indicated times, decapitated, and brain homogenates prepared. Cyclase activity in nonirradiated control preparations was 75 pmoles/mg protein/min in the absence of NaF and 250 pmoles/mg proteins/min in the presence of 10 mM NaF. (The above curve is a composite of data obtained under both sets of conditions, since there was no significant difference between them when plotted as percent of control.) Control phosphodiesterase activity was 10 nmoles/mg protein/min. (From Schmidt et al., 1972*b*).

Paul, Pauk, and Ditzion (1970) had reported that amphetamine had no effect on whole brain levels of cyclic AMP in rats killed either by decapitation or freezing. Since amphetamine has been shown to stimulate the release of newly synthesized norepinephrine in the brain (Sulser and Bush, 1971), and since norepinephrine was known to increase the level of cyclic AMP in rat brain slices *in vitro* (see below), it seemed reasonable to suppose that amphetamine should affect brain cyclic AMP levels *in vivo*. We therefore decided to reinvestigate this question using the MI technique. A dose of amphetamine sufficient to produce clear-cut CNS stimulation (3 mg/kg) was administered

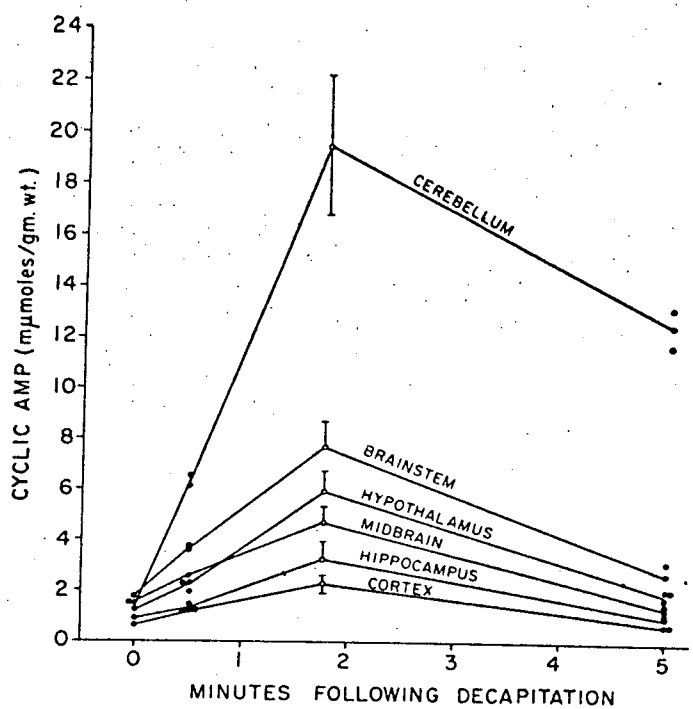

FIG. 3. Cyclic AMP levels in various brain areas after decapitation. Rats were decapitated, and the heads exposed to 30 sec of MI at the indicated times. Zero time values were obtained from rats exposed to MI *before* decapitation. (From Schmidt et al., 1971)

intraperitoneally, and the rats were killed at various times between 5 and 60 min after injection. At none of these times, however, did amphetamine produce a change in the level of cyclic AMP in any of the four brain areas examined (Table 2). A higher dose of amphetamine (10 mg/kg) was also without effect.

The ability of norepinephrine to raise the level of cyclic AMP in rat brain slices *in vitro* has been observed by many investigators (Kakiuchi and Rall, 1968; Schmidt, Palmer, Dettbarn, and Robison, 1970; Palmer, Robison, and Sulser, see also the review by Daly, Huang, and Shimizu in this volume). Since a drug which releases norepinephrine did not appear to increase cyclic AMP concentrations, we attempted to examine the effect of norepinephrine more directly by simulating *in vivo* the *in vitro* experiments. To do this we injected norepinephrine directly into the brain by means of a cannula placed in the lateral ventricle, using the technique developed by Robinson, Hengeveld, and de Balbian Verster (1969). It seemed likely that the intraventricular injection of norepinephrine would increase the level of cyclic AMP *in vivo* as it does when added *in vitro* to brain slices. However, as with amphetamine,

TABLE 2. *Lack of effect of amphetamine on cyclic AMP levels in various areas of the rat brain*[a]

Time after injection (min)	Cyclic AMP (nmoles/g)			
	Cerebellum	Brainstem	Midbrain	Cortex
5 control	1.80 ± 0.04	1.77 ± 0.08	1.35 ± 0.04	0.89 ± 0.11
amphetamine	1.81 ± 0.05	1.69 ± 0.10	1.42 ± 0.07	0.81 ± 0.05
15 control	2.36 ± 0.08	2.06 ± 0.01	1.62 ± 0.07	1.00 ± 0.02
amphetamine	2.11 ± 0.02	2.00 ± 0.06	1.51 ± 0.09	0.93 ± 0.06
30 control	1.80 ± 0.04	1.77 ± 0.08	1.35 ± 0.04	0.89 ± 0.11
amphetamine	1.80 ± 0.07	1.70 ± 0.14	1.32 ± 0.11	0.86 ± 0.08
60 control	1.98 ± 0.18	1.97 ± 0.08	1.47 ± 0.12	0.84 ± 0.06
amphetamine	1.77 ± 0.11	1.89 ± 0.11	1.51 ± 0.14	0.72 ± 0.08

[a]Rats received an intraperitoneal injection of 3 mg/kg amphetamine or saline. Exposure to 30 sec of MI followed at the indicated times. Each value represents the mean ± S. E. M. of three to four separate determinations. (From Schmidt et al., 1972*b*)

the results were completely negative. A large dose of norepinephrine (5 μg failed to change cyclic AMP concentration in any of the brain areas studie when the rats were killed either 5 or 30 min after the injection. A smaller dos (50 ng) was equally ineffective (Schmidt, Hopkins, Schmidt, and Robison 1972*b*).

III. DISCUSSION

The failure of amphetamine and especially of intraventricular norepinephrine to alter brain cyclic AMP levels was surprising, and could call into question the validity of MI as a method of tissue fixation. There are a number of points which could be considered here. First, the MI technique does appear to represent an improvement over freezing by liquid nitrogen, since MI inactivates enzymes much more rapidly, especially in deep brain structures. A further advantage not previously mentioned is that irradiated brains tend to separate along natural anatomical boundaries, so that they are actually easier to dissect than fresh brains. For these reasons, MI appears to be preferable to freezing as a method of tissue fixation.

However, the MI technique used in these studies is still far from ideal, the ideal in this case being a method that would instantaneously and irreversibly inactivate all enzymes throughout the brain with no damage to cell structure. The chief disadvantage of our present technique is that it is probably still too slow, since a great deal could happen during the 20 sec that elapse between the onset of MI and complete enzyme inactivation. There might first be time for a sudden massive release of endogenous norepinephrine or other factors

in response to MI, thus leading to a rise in cyclic AMP that might mask an effect of exogenous norepinephrine. Second, there must be a short but finite period between the onset of MI and the beginning of protein denaturation during which the activities of many enzymes will be greater than normal. Third, even after enzyme activities begin to decrease (which is clearly earlier than 5 sec in the case of cyclase and phosphodiesterase), an imbalance may occur within the cell if opposing enzymes are inactivated at unequal rates. Because of the various regulatory influences to which these enzymes might be subject *in vivo*, the precise nature of this imbalance cannot be predicted from the simple type of *in vitro* study illustrated in Fig. 2. Some of these possibilities can be and are being tested experimentally, but, from the practical point of view, perhaps the best solution would be to develop a more powerful unit which will do in milliseconds what our present unit does now in seconds. We are attempting to develop such a unit, but how close we can come to the previously stated ideal remains to be seen.

In the meantime, there are other considerations which lead us to believe that our present technique is valid, i.e., that the cyclic AMP values so obtained are reasonably close to the levels actually existing in the brain prior to the onset of MI. First, the post-decapitation rise in cyclic AMP was readily detected by the MI technique, and we have therefore felt justified in assuming that drug-induced changes of similar magnitude would also have been detected, had they occurred. Second, we have found (J. Blumberg, *unpublished observations*) that glucagon-induced increases in hepatic cyclic AMP and ACTH-induced increases in adrenal cyclic AMP are also readily detectable in irradiated animals. Since the temperatures in these peripheral organs probably rise less rapidly than in the brain, under the conditions of our experiments, this further supports our view that had amphetamine or norepinephrine produced an overall increase in the level of cyclic AMP in any of the brain areas we examined, we would have been able to detect it.

An alternate possibility to explain the lack of a norepinephrine effect *in vivo* might therefore be considered. The large changes in cyclic AMP which we and others have observed in rat brain slices incubated *in vitro* in the presence of norepinephrine may have to some extent been artifactual. An unwritten assumption (by us and probably others) has been that these changes primarily reflect changes occurring in neurons. Now, however, at least two groups of investigators (Gilman and Nirenberg, 1971; Clark and Perkins, 1971) have observed extraordinary increments in cyclic AMP in cultured *glial* cells in response to norepinephrine. Furthermore, Gilman and Nirenberg were unable to see any effect of norepinephrine on neuroblastoma cells, even though these cells did respond to certain other agents, such as the prostaglandins. Cohen (1962) reported that extensive histologic alterations occurred in neurons of brain slices incubated in oxygenated Krebs-Ringer glucose medium, whereas glial cells survived the incubation without apparent effect. Cyclic AMP levels

in brain slices do decrease in the course of incubation, eventually reaching levels which provide the baseline for most of the studies which we and others have reported. It now seems possible that most of the norepinephrine-induced increments observed to occur in these preparations were occurring in glial cells against a background of defective neurons. It further seems possible that such large changes might not occur if the glia were in proper contact with functional neuronal cells.

IV. SUMMARY

Microwave irradiation (MI) appears to be a useful method of fixing tissue *in situ* for the measurement of cyclic AMP and other heat-stable compounds. Using a commercial microwave oven as the source of MI, we have applied this method to the study of cyclic AMP levels in discrete regions of the rat brain *in situ*. Death occurs within 5 sec of exposure to MI, at which point the temperature of the thalamus is approximately 55°C. Continued exposure leads to a linear increase in temperature reaching 90°C after 30 sec. Tissue levels of cyclic AMP remain constant after 20 sec of MI, beyond which time neither adenylyl cyclase nor phosphodiesterase can be detected in tissue homogenates. This is slower than might be desired, but considerably faster than other known methods. Improvements such as increased power and the ability to focus the irradiation may lead to a method more nearly approaching the ideal.

Irradiated brains can be easily removed from the skull and dissected into their component parts. Cyclic AMP levels were highest in the cerebellum and brainstem, intermediate in the hypothalamus and midbrain, and lowest in the cortex and hippocampus. The cerebellum was found to contribute disproportionately to the post-decapitation rise in cyclic AMP. Neither amphetamine nor the intraventricular injection of norepinephrine had any detectable effect on cyclic AMP levels in any of the brain areas studied. This was surprising because norepinephrine had previously been shown to produce large increases in the level of cyclic AMP in rat brain slices *in vitro*. Some possible reasons for this apparent discrepancy were discussed.

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REFERENCES

Breckenridge, B. McL. (1964): The measurement of cyclic adenylate in tissues. *Proceedings of the National Academy of Sciences*, 52 : 1580–1586.

Clark, R. B., and Perkins, J. P. (1971): Increase of cyclic AMP in cultured human glial cells mediated by norepinephrine and histamine. *Pharmacologist*, 13 :256.
Cohen, M. M. (1962): The effect of anoxia on the chemistry and morphology of cerebral cortex slices *in vitro*. *Journal of Neurochemistry*, 9 :337–344.
Gilman, A. G., and Nirenberg, M. (1971): Effect of catecholamines on adenosine 3',5'-monophosphate concentrations of clonal satellite cells of neurons. *Proceedings of the National Academy of Sciences*, 68 :2165–2168.
Jongkind, J. F., and Bruntink, R. (1970): Forebrain freezing rates and substrate levels in decapitated rat heads. *Journal of Neurochemistry*, 17 :1615–1617.
Kakiuchi, S., and Rall, T. W. (1968): The influence of chemical agents on the accumulation of adenosine 3',5'-phosphate in slices of rabbit cerebellum. *Molecular Pharmacology*, 4 :367–378.
O'Dea, R. F., Haddox, M. K., and Goldberg, N. D. (1971): Cyclic AMP-kinase complex: Nonreactivity with phosphodiesterase and characteristics of interaction. *Federation Proceedings*, 30 : 219 Abs.
Palmer, E. C., Robison, G. A., and Sulser, F. (1971): Modification by psychotropic drugs of the cyclic adenosine monophosphate response to norepinephrine in rat brain. *Biochemical Pharmacology*, 20 :236–239.
Paul, M. I., Pauk, G. L., and Ditzion, B. R. (1970): The effect of centrally acting drugs on the concentration of brain adenosine 3',5'-monophosphate. *Pharmacology*, 3 :148–154.
Richter, D., and Dawson, R. M. C. (1948): Brain metabolism in emotional excitement and in sleep. *American Journal of Physiology*, 154 :73–79.
Robinson, C. A., Hengeveld, C. A., and de Balbian Verster, F. (1969): Improved polyethylene cannulation technique. *Physiology and Behavior*, 4 :123–124.
Robison, G. A., Butcher, R. W. Øye, I., Morgan H. E., and Sutherland, E. W. (1965): The effect of epinephrine on adenosine 3', 5'-phosphate levels in the isolated perfused rat heart. *Molecular Pharmacology*, 1 :168–177.
Schmidt, D. E., Speth, R. C., Welsch, F., and Schmidt, M. J. (1972*a*): The use of microwave irradiation in the determination of acetylcholine in the rat brain. *Brain Research*, 38 :377–389.
Schmidt, M. J., Palmer, E. C., Dettbarn, W.-D., and Robison, G. A. (1970): Cyclic AMP and adenyl cyclase in the developing rat brain. *Developmental Psychobiology*, 3 :53–67.
Schmidt, M. J., Schmidt, D. E., and Robison, G. A. (1971): Cyclic adenosine monophosphate in brain areas: Microwave irradiation as a means of tissue fixation. *Science*, 173 :1142–1143.
Schmidt, M. J., Hopkins, J. T., Schmidt, D. E., and Robison, G. A. (1972*b*): Cyclic AMP in the brain: effects of amphetamine and norepinephrine assessed through the use of microwave irradiation as a means of tissue fixation. *Brain Research, in press.*
Stavinoha, W. B., Pepelko, B., and Smith, P. W. (1970): Microwave radiation to inactivate cholinesterase in the rat brain prior to analysis for acetylcholine. *Pharmacologist*, 12 :257.
Sulser, F., and Bush, E. S. (1971): Effect of drugs on amines in the CNS. *Annual Review of Pharmacology*, 11 :209–230.
Takahashi, R., and Aprison, M. H. (1964): Acetylcholine content of discrete areas of the brain obtained by a near-freezing method. *Journal of Neurochemistry*, 11 :887–898.
Weiss, B., and Costa, E. (1968): Regional and subcellular distribution of adenyl cyclase and 3', 5'-cyclic nucleotide phosphodiesterase in brain and pineal gland. *Biochemical Pharmacology*, 17 :2107–2116.
Wollenberger, A., Ristau, O., and Schoffa, G. (1960): Eine einfache technik der extrem schnellen abkühlung grösserer gewebestücke. *Pflügers Archive für die Gesamte Physiologie*, 270 :399–412.

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Psychopharmacological Agents and the Cyclic AMP System of Rat Brain

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I. INTRODUCTION

Of the many avenues one might take to determine the etiology and pathogenesis of endogenous psychoses, one promising approach would be to study the effect of psychotropic drugs on the basic biochemical mechanisms involved in regulating brain functions. There is a large body of evidence suggesting that adenosine 3',5'-monophosphate (cyclic AMP) plays an important role in the central nervous system (Weiss and Kidman, 1969) and that psychotropic drugs may act by affecting the concentration or the actions of cyclic AMP in brain. For example, Kakiuchi and Rall (1968*a*) showed that chlorpromazine treatment antagonized the rise of cyclic AMP in rabbit brain following decapitation, and Palmer, Robison, and Sulser (1971) and Uzunov and Weiss (1971) have reported that several tranquilizers blocked the norepinephrine-induced increase in the concentration of cyclic AMP in rat brain slices.

In addition, brain has particularly high activities of adenylate cyclase and cyclic nucleotide phosphodiesterase (Butcher and Sutherland, 1962; Sutherland, Rall, and Menon, 1962; Weiss and Costa, 1968*a*) and possesses relatively high concentrations of cyclic AMP (Ebadi, Weiss, and Costa, 1971*a*). These enzymes as well as the concentration of cyclic AMP itself are unequally distributed throughout the brain and are associated with synaptic elements [(De Robertis, Rodriguez de Lores] Arnatiz, Alberici, Butcher, and Sutherland, 1967; Weiss and Costa, 1968*a*; Ebadi et al., 1971*b*). Moreover, cyclic AMP has been shown to produce various effects on neuronal tissue. It activates a protein kinase from brain (Miyamoto, Kuo, and Greengard, 1969), it inhibits the firing of cerebellar Purkinje cells when administered microiontophoretically (Siggins, Hoffer, and Bloom, 1969), and it induces a variety of behavioral

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Jongkind, J. F., and Bruntink, R. (1970): Forebrain freezing rates and substrate levels in decapitated rat heads. *Journal of Neurochemistry*, 17 :1615–1617.
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