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Author	木村, 都(Kimura, Miyako) 笠原, 純子(Kasahara, Junko) 小池, 敦子(Koike, Atsuko)
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Fluctuation of Cyclic AMP and Cyclic GMP Levels in the Brain and in the Uterus Endometrium during the Estrous Cycle of Rats

Miyako KIMURA, Junko KASAHARA and Atsuko KOIKE

木村　都，笠原純子，小池敦子

Contents of cyclic AMP and cyclic GMP in seven regions of the brain and the uterus endometrium (UEM) were determined during the estrous cycle in intact female rats. Effects of 17β-estradiol (EST) and progesterone (PRG) on these cyclic uncleotides contents in the brain and UEM were also studied in ovariectomized rats. Increased cyclic AMP content was observed in proestrus (P) and estrus II (E II) stages in most regions of the brain. A significant increase in cyclic GMP content was observed in cerebellum, midbrain and hippocampus in E II stage. However, the magnitude of the change was much smaller than that of cyclic AMP. In UEM, cyclic AMP content was increased in P stage and was decreased in E II stage. Cyclic GMP content increased in P and estrus I (E I) stages. Thus, the ratio of (cyclic AMP) / (cyclic GMP) in UEM fell in P, E I, E II and metestrus stages showing a U-shaped curve. EST induced significant increases in cyclic AMP content in hypothalamus, midbrain and UEM, and in cyclic GMP content in cortex, hypothalamus and striatum. PRG produced no significant changes in these cyclic nucleotides contents in the brain, while in UEM, PRG produced significant decrease in cyclic AMP and an increase in cyclic GMP content. Thus, the ratio of (cyclic AMP) / (cyclic GMP) was significantly increased by EST and decreased by PRG in UEM. These data indicate that the cyclic AMP content in the brain fluctuates during the estruos cycle and that EST appears to be involved at least in part in this fluctuation. In UEM, it is suggested that the changes in cyclic AMP and cyclic GMP refer to the action of EST and PRG.

INTRODUCTION

It is generally accepted that the ovarian cycle in mammals is related to the secretion of hormones in hypothalamo-hypophyseo-gonadal system. However, it remains to be clarified how 17β-estradiol (EST) act on the development of estrous cycle and on the synthesis and release of gonadotropin (GTH) or gonadotropin releasing hormone. It has been suggested that there is a link between this feedback regulation and the action of central biogenic amines[1-4]. Weissman et al.[5] reported that EST increased significantly cyclic AMP formation in the hypothalamus of rat *in vitro*, but this action might be indirect because the increase was observed only 40～50 min after the incubation. Therefore, it is considered that the contents of cyclic nucleotides in the brain, especially in hypothalamus may change with the ovarian cycle. In this study,

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to investigate the relationship between cyclic nucleotides and estrous cycle, the cyclic AMP and cyclic GMP contents during the cycle in seven regions of the brain and in uterus endometrium (UEM), which is the target organ of ovarian hormones, were determined. Effects of EST and progesterone (PRG) on the contents of cyclic AMP and cyclic GMP were also studied in ovariectomized rats.

MATERIALS AND METHODS

Female Sprague-Dawley rats were housed individually for over 2 weeks in a room with constant temperature 23±1 °C and 12-hr lighting cycle and had free access to standard rat chow (CE-2, Nippon Clea) and water. Estrous cycle was surveyed by daily vaginal smears, and only the rats exhibiting a normal cycle were used for the study at about 12 weeks of age.

The estrous cycle was classified into the stages by the findings of smears; diestrus (D, having many leucocytes and some epithelial cells), proestrus (P, having only epithelial cells), estrus I (E I, having epithelial cells and cornified cells), estrus II (E II, having only cornified cells) and metestrus (M, leucocytes beginning to appear after E II stage).

After the confirmation of the stage of cycle, rat was killed by exposing the head to a microwave beam (1.3 KW, 2450 MHz) for 3 sec to inactivate enzymes in the brain. The brain was dissected into seven regions by the method of Glowinski and Iversen[6]; hypothalamus, striatum, midbrain, hippocampus, cortex, cerebellum and medulla oblongata. The uterus was exposed to microwave radiation for 0.5 sec immediately after the removal to inactivate adenylate cyclase in the uterus. The endometrium was scraped off and collected. The tissues were homogenized in normal saline with a Teflon homogenizer. Protein was removed by the addition of trichloroacetic acid and centrifugation at 600×g for 15 min. The supernatant was rinsed three times with water-saturated ether and evapolated to dryness under vacuum. The residue was dissolved in 0.3 ml of distilled water for the assay of cyclic AMP and cyclic GMP by radioimmunoassay using the assay kits (Yamasa). A portion of the above homogenate was used for protein assay by the method of Lowry et al.[7]. The concentrations of cyclic nucleotides were expressed as p mole per mg of protein.

To study effects of ovarian hormones on the cyclic nucleotides contents in the brain and UEM, rats were ovariectomized under ether anesthesia and used 2 weeks after the surgery. EST (0.2 mg/kg, in saline containing 2 % ethanol), PRG (4 mg/kg, in the same vehicle) or the vehicle were administrated (i. p.) at 2hr before killing.

Results are presented as the mean ± S.E. Significant difference among the groups was assessed by the multiple "*t*" test.

RESULTS

1) Cyclic AMP and cyclic GMP contents in the brain and UEM during the estrous cycle.

Significant increases in cyclic AMP content were recognized in cerebellum in E II stage, in cortex in E II and M stages, in midbrain in E II and M stages and in medulla oblongata in E II stage ($p < 0.05$ or 0.01) (Fig. 1).

The content of cyclic GMP in the brain was about one-tenth of cyclic AMP (Fig. 2). Significant increases in cyclic GMP content were observed in cerebellum in E II stage, in midbrain in E I and E II stages, and in hippocampus in E I, E II and M stages ($p < 0.05$). However, the magnitude of the change in cyclic GMP content in the brain was less than that in cyclic AMP.

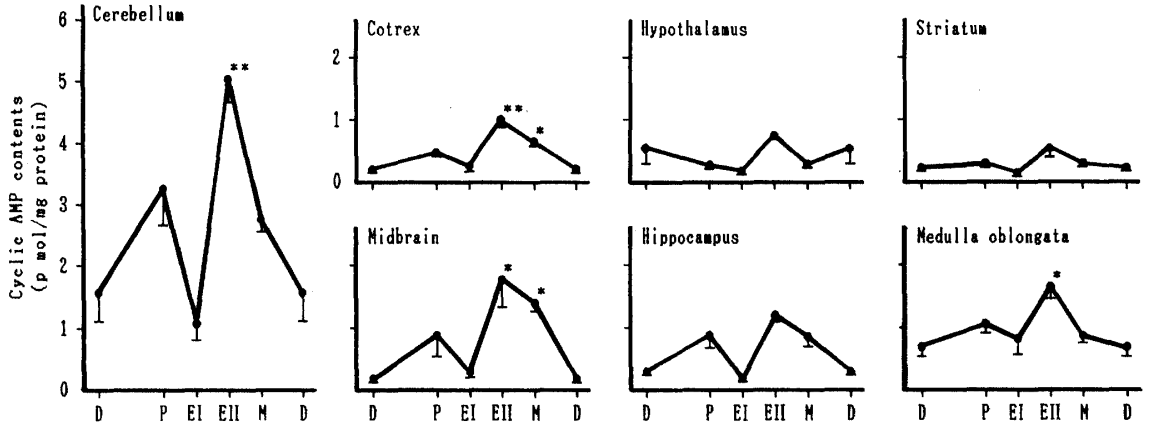

Fig. 1 Changes in cyclic AMP contents in the rat brain during the estrous cycle.
Each point and bar represent the mean±S.E. of four rats. Significantly different from the value in the diestrus stage (D), * : $p < 0.05$, ** : $p < 0.01$.
P; proestrus, EI; estrus I, EII; estrus II; M; metestrus

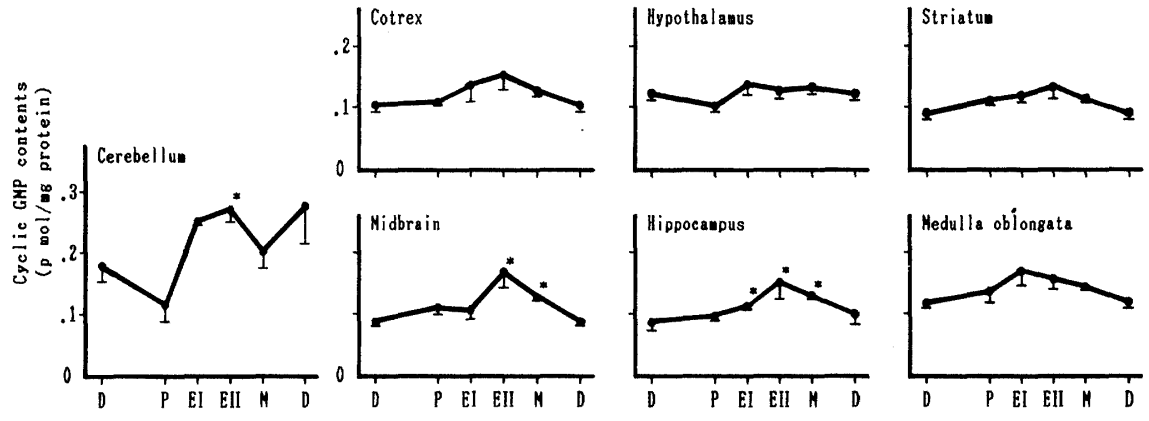

Fig. 2 Changes in cyclic GMP contents in the rat brain during the estrous cycle.
Each point and bar represent the mean±S.E. of four rats. Significantly different from the value in the diestrus stage (D), * : $p < 0.05$, ** : $p < 0.01$.
P; proestrus, EI; estrus I, EII; estrus II; M; metestrus

In UEM, the change of cyclic AMP content during the cycle was distinct from that in cyclic GMP content (Fig. 3). The ratio of (cyclic AMP) / (cyclic GMP) fell in P, E I, E II and M stages, showing a U-shaped curve. The ratio in D stage was about 5-fold of that in E I and E II stages.

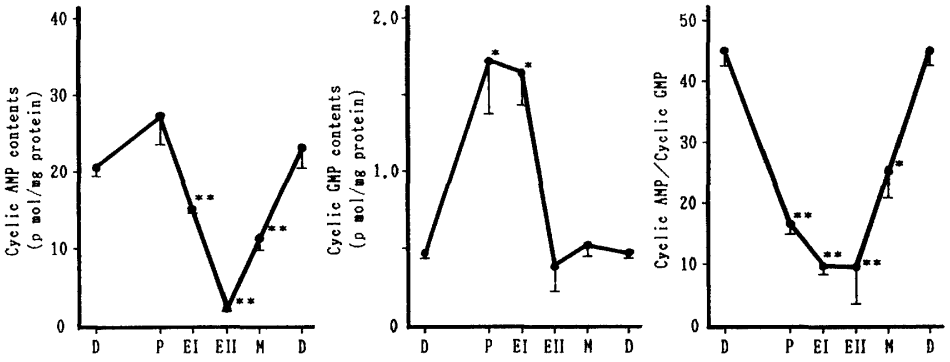

Fig. 3 Changes in cyclic nucleotides and (cyclic AMP) / (cyclic GMP) ratio in uterus endometrium during the estrous cycle. Each point and bar represent the mean±S.E. of four rats. Significantly different from the value in the diestrus stage (D), * : $p < 0.05$, ** : $p < 0.01$.
P; proestrus, EI; estrus I, EII; estrus II; M; metestrus

2) Effects of EST and PRG on cyclic AMP and cyclic GMP contents in the brain and UEM in ovariectomized rats.

The contents of cyclic AMP and cyclic GMP in the brain in ovariectomized rats were comparable to those in D stage in intact rats. EST produced a significant increase in cyclic AMP content in hypothalamus and midbrain ($p < 0.05$), and tended to increase in other regions of the brain. On the other hand, PRG did not affect the content of cyclic AMP in the brain (Fig. 4). Cyclic GMP content in cortex, hypothalamus and striatum was increased significantly by EST ($p < 0.05$), while PRG produced no significant effect on cyclic GMP content in the brain (Fig. 5).

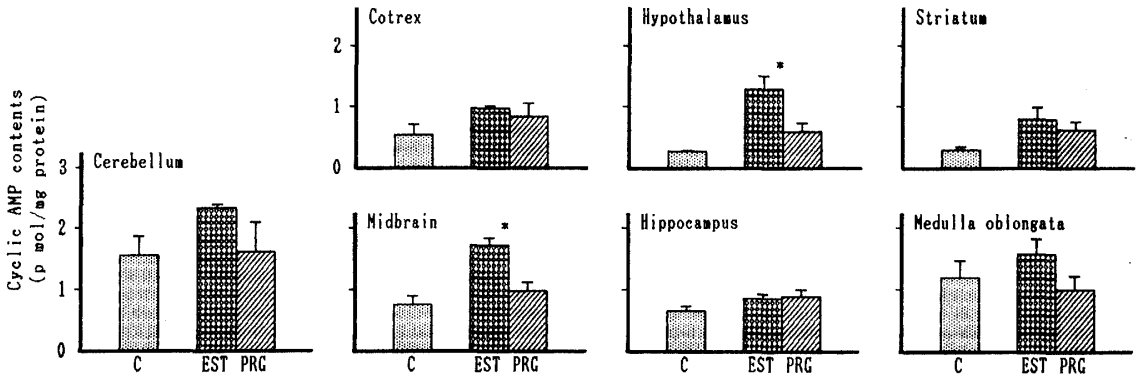

Fig. 4 Effects of 17β-estradiol or progesterone on the brain contents of cyclic AMP in ovariectomized rats. Vertical bars indicate the mean±S.E. of four rats.
* shows the significant difference from the value in vehicle treated control (C) at $p < 0.05$.
EST; rats injected 17β-estradiol 0.2mg/kg, PRG; rats injected progesterone 4 mg/kg

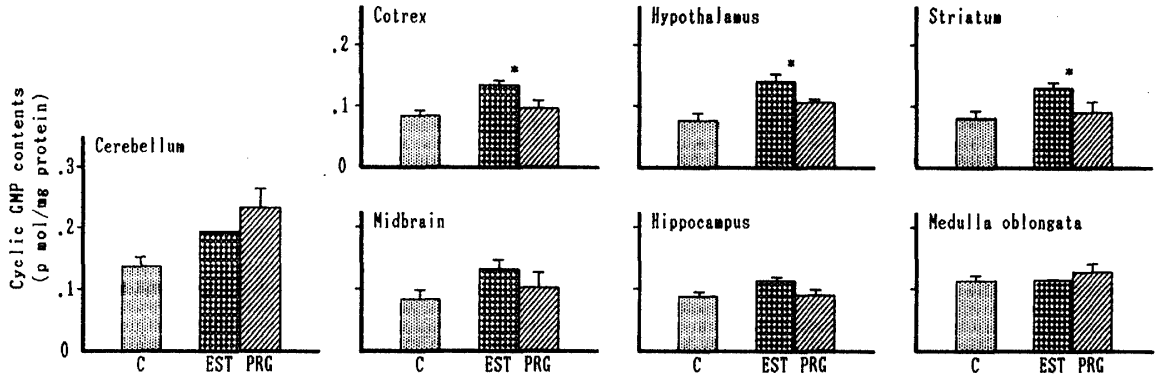

Fig 5 Effects of 17β-estradiol or progesterone on the brain contents of cyclic GMP in ovariectomized rats. Vertical bars indicate the mean±S.E. of four rats.
* shows the significant difference from the value in vehicle treated control (C) at $p < 0.05$.
EST; rats injected 17β-estradiol 0.2 mg/kg, PRG; rats injected progesterone 4 mg/kg

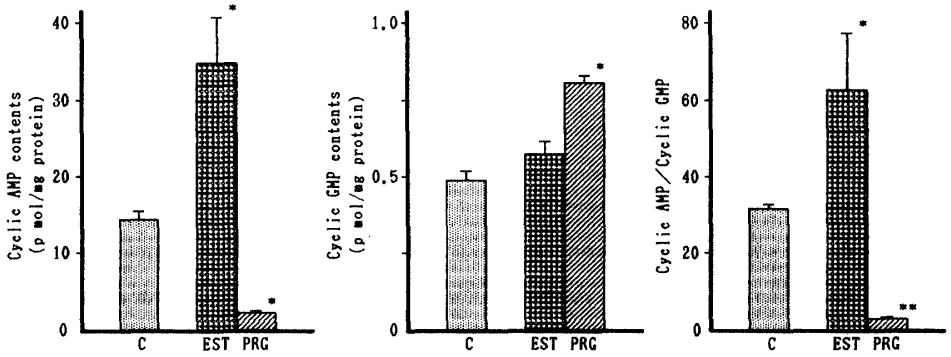

Fig 6 Effects of 17β-estradiol or progesterone on the contents of cyclic AMP, cyclic GMP and (cyclic AMP)/(cyclic GMP) ratio in the uterus endometrium of ovariectomized rats.
Vertical bars indicate the mean±S.E. of four rats.
*, ** shows that significant difference from the value in vehicle treated control (C) at $p < 0.05$, $p < 0.01$, respectively.
EST; rats injected 17β-estradiol 0.2 mg/kg, PRG; rats injected progesterone 4 mg/kg

In UEM, the cyclic nucleotides in the ovariectomized rats were nearly equal to those in D stage in intact rats (Fig. 6). Cyclic AMP content was increased significantly by EST ($p < 0.05$), while it was decreased by PRG ($p < 0.05$). PRG produced a significant increase in cyclic GMP content in UEM ($p < 0.05$). Thus, the ratio of (cyclic AMP)/(cyclic GMP) was increased by EST ($p < 0.05$), while it was depressed to one-fifteenth by PRG ($p < 0.01$).

The results were showed a similar tendency when expressed on tissue basis, though the protein contents in UEM and brain varied slighty by the stages of cycle, ovariectomy or hormone injection (data were not shown).

DISCUSSION

It is generally accepted that cyclic AMP is implicated as second messenger in the actions of catecholamines and some hormones. Gunaga and Menon[1] described that the increase in the cyclic AMP by EST in the immature rat hypothalamus was blocked by adrenergic antagonists *in vivo* and *in vitro*. In addition, it has been reported that the content of norepinephrine decreases and the dopamine content increases in diencephalon in P stage in 4-day cycle rat[8], and that monoamine oxidase (MAO) activity decreases in the brain, especially in hypothalamus in P stage[9,10]. Nakano et al.[11] found that cyclic GMP decreased rapidly by LH–RH and induced LH release from the cultured female rat anterior pituitary cells, and proposed that cyclic GMP might act as an intracellular mediator in the process of GTH release stimulated by LH–RH. Motohashi[12] observed LH release by LH–RH in the estrus stage in the 4-day cycle rats, but not in the D stage. These findings suggest that the cyclic nucleotides in the brain may fluctuate during the estrous cycle relating to the concentrations of the brain catecholamines, and plasma LH, FSH, EST and PRG. The fluctuation of brain cyclic nucleotides observed in the present study coincided with the time of sharp changes of LH, FSH, EST and PRG in plasma[13], and catecholamines and MAO activity in the brain. As it is accepted that the ovulation occurrs in the last stage of P, we consider that cyclic nucleotides may play an important role in the estrous cycle.

A larger discrepancy in content ratio of (cyclic AMP)/(cyclic GMP) is frequently observed, because the cyclic GMP content is very lower than cyclic AMP content in most mammalian tissues and when one of both nucleotides increases, the other decreases[15]. The ratio of (cyclic AMP)/(cyclic GMP) in UEM changed largely with the cycle, because the pattern of fluctuation of cyclic AMP was different from that of cyclic GMP during the cycle, however, this phenomenon did not recognized in brain. The results indicate that the fluctuation of cyclic nucleotides and the ratio of (cyclic AMP)/(cyclic GMP) might be related to the historlogical chages in UEM, which was the state of maximal hypertrophy in E II.

After the ovariectomy, the contents of cyclic AMP and cyclic GMP in the brain and UEM were retained at the level of D stage in intact rats. EST elevated cyclic AMP level largely and cyclic GMP slightly in the brain and UEM. The effects of ovariectomy and EST on cyclic AMP in UEM are similar to the report by Szego and Davis[14] that cyclic AMP in uterus was reduced to one half by ovariectomy and recovered to the normal level within 5 min after the EST injection (10 g/kg, i. v.).

The present results demonstrate that cyclic AMP contents in the brain and UEM vary during the estrous cycle and fluctuate largely from stage P to stage E II corresponding to the period from LH surge to the ovulation, and that the increase in cyclic AMP content was evidently stimulated with EST. It is suggested that cyclic AMP may be act as an important regulatory agent in the control of estrous cycle in

brain, although it is not affirm whether the changes observed in cyclic nucleotides during the cycle were directly resulted by the plasma level of EST. The studies used anti estrogens is necessary in order to assess whether the action of EST in increasing the content of cyclic AMP mediated through the estrogenic receptors.

It is suggested that the chages in cyclic AMP and cyclic GMP refer to the action of EST and PRG on the UEM. It has been considered that the histological changes of UEM were based on the ovarian hormones, being different from the case in brain, but further work is needed to clarify whether the fluctuation of the ratio of (cyclic AMP)/(cyclic GMP) directly leads to the histological change of the UEM during the cycle.

REFERENCES

1) Gunaga, K.P. and Menon, K.M.J. : Effect of catecholamines and ovarian hormones on cyclic AMP accumulation in rat hypothalamus. Biochem. Biophys. Res. Commun. **54**, 440~448, 1973
2) Gunaga, K.P., Kawano, A. and Menon, K.M.J. : In vitro effect of estradiol benzoate on the accumulation of adenosine 3',5'-cyclic monophosphate in the rat hypothalamus. Neuroendocrinol. **16**, 273~281, 1974
3) Weissman, B.A. and Skolnick, P. : Stimulation of adenosine 3',5'-monophosphate formation in incubated rat hypothalamus by estrogenic compounds : Relationship to biologic potency and blockade by antiestrogens. Neuroendocrinol. **18**, 27~32, 1975
3) Endersby, C.A. and Wilson, C.A. : The effect of ovarian steroids on the accumulation of ^{3}H-labelled monoamines by hypothalamic tissue in vitro. Brain Res. **73**, 321~331, 1974
5) Weissman, B.A., Daly, J.W. and Skolnick, P. : Diethylstilbestrol-elicited accumulation of cyclic AMP in incubated rat hypothalamus. Endocrinol. **97**, 1559~1566, 1975
6) Glowinski, J. and Iversen, L.L. : Regional studies of catecholamines in the rat brain~1. The disposition of [^{3}H] norepinephrine, [^{3}H] dopamine and [^{3}H] dopa in various regions of the brain. J. Neurochem. **13**, 655~669, 1966
7) Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. : Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**, 265~276, 1951
8) Kurachi, K. : Amines in the hypothalamus and their regulation of gonadal function. Metabolism. **9**, 458~467, 1972 (In Japanese)
9) Holzbauer, M. and Youdim, M.B.H. : The oestrous cycle and monoamine oxidase activity. Br. J. Pharmac. **48**, 600~608, 1973
10) Kimura, M. and Nakamura, E. : Studies on monoamine oxidase (IV) : Monoamine oxidase activity in rat brain and estrous cycle. Jap. J. Pharmac. **28**. 163p, 1978
11) Nakano, H., Fawcett, C.P., Kimura, F. and MaCann, S.M. : Evidence for the involvement of guanosine 3',5'-cyclic monophosphate in the regulation of gonadotropin release. Endocrinol. **103**, 1527~1533, 1978
12) Motohashi, T. : A study on the regulatory mechanism of hypothalamo-hypophseo-ovarian system in estrous cycles of rats : Changes on responsiveness of the anterior pituitary gland to synthetic LH–RH.

Folia endocrinol. Jap. **51**, 661～675, 1975 (In Japanese)

13) Butcher, R.L., Collins, W.E. and Fugo, N.W. : Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-days estrous cycle of the rat. Endocrinol. **94**, 1704～1708, 1974

14) Szego, C.M. and Davis, J.S. : Adenosine 3',5'-monophosphate in rat uterus : Acute elevation by estrogen. Proc. Nat. Acad. Sci. USA. **58**, 1711～1718, 1967

15) Mao, C.C. and Guidotti, A. : Simultaneous isolation of adenosine 3',5'-cyclic monophosphate (cAMP) and guanosine 3',5'-cyclic monophosphate (cGMP) in small tissue samples. Analytical biochemistry **59**, 63～68, 1974