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EFFECTS OF ERGOTS ON ADENYLATE CYCLASE ACTIVITY IN THE CORPUS STRIATUM AND PITUITARY

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Summary

Adenylate cyclase activity was determined in homogenates of the corpus striatum and pituitary gland. Dopamine and several ergots stimulated cyclic AMP synthesis in the striatum, but no stimulation was seen in the pituitary gland. None of the ergots tested were as active as dopamine itself, and all were able to partially inhibit the dopamine-induced activation of adenylate cyclase. Lergotrile, a simple ergoline derivative which displays dopamine agonist activities in the pituitary gland and striatum, did not stimulate adenylate cyclase in either tissue. These findings show that the in vivo dopaminergic activity of ergots is not reflected in the dopamine-dependent adenylate cyclase assay using either the corpus striatum or the pituitary gland. It is suggested that those dopamine receptors in the pituitary gland which mediate prolactin release are not associated with adenylate cyclase.

Ergot alkaloids have a diverse, complex pharmacological profile. Ergot-like compounds interact with alpha receptors (1), serotonin receptors (2), and dopamine receptors (3) and exert direct effects on some smooth muscle systems (4). Clinically these agents produce hallucinations, decrease serum prolactin levels, contract the uterus, aid in the treatment of migraine headaches and are beneficial in Parkinsonism (5). Many of the clinical effects of ergots are compatible with the dopaminergic activity of these compounds and several ergots have shown dopamine agonist activity in animal tests. Direct iontophoresis of agroclavine stimulates dopamine-sensitive cortical neurons in the rat (6) and injection of ergometrine into the nucleus accumbens results in a dopamine-like enhancement of motor activity (7). Ergots protect against audiogenic seizures (8), produce stereotyped behavior in rats (9), emesis in dogs (10), contralateral rotation in 6-hydroxydopamine lesioned rats (11), and alleviate tremors produced by tegmental lesions in monkeys (12). Reduced turnover of dopamine in the striatum and limbic system (13) and antagonism of dopamine binding in several brain regions have also been reported for a number of ergots (3). Dopamine agonist activity

of ergots has been demonstrated in the retina (14), the nucleus accumbens (7), and the pituitary gland (15). It seems clear from the multiplicity and diversity of the experiments to date that the dopamine agonist property of the ergots is real and therapeutically important.

How the ergots exert dopamine agonist activity within cells is not known, although alterations in the concentration of cyclic AMP may be involved in the mechanism of action of ergots. Methylxanthines enhance the rotational behavior produced by ergocornine and 2-Br-α-ergocriptin (CB-154) which suggests that inhibition of cyclic AMP phosphodiesterase can potentiate ergot activities (16). That the rotational model involves cyclic AMP was supported by the fact that apomorphine elevated striatal cyclic AMP levels while enhancing rotational behavior (17).

Kebabian et al. (18), proposed that the dopamine receptor and dopamine sensitive adenylate cyclase in the corpus striatum were one and the same, suggesting that synthesis of cyclic AMP might be the common means by which dopamine exerts its effects in target tissues. Many studies have supported this contention and have demonstrated that measurement of adenylate cyclase is a useful means of biochemically characterizing the dopamine agonist or antagonist activity of compounds (19). One would assume that ergots would be active stimulants of striatal adenylate cyclase, however, at present only LSD has been tested in this system and its activity was not as expected. Low concentrations of LSD stimulated cyclic AMP synthesis, but never to the extent that dopamine stimulated activity; and higher concentrations of LSD inhibited dopamine-stimulated cyclic AMP synthesis (20).

The present studies were undertaken to explore the effects of several structurally different ergot-like compounds on adenylate cyclase of the corpus striatum and pituitary gland, two tissues where ergots exert marked dopamine-like effects both in vivo and in vitro.

Methods and Materials

Adenylate cyclase activity was assayed in homogenates of pituitary glands or brain areas by monitoring the conversion of ^{32}P-α-ATP to ^{32}P-cyclic AMP. Purification of cyclic AMP was accomplished through sequential chromatography using neutral alumina and Dowex 1. Male Wistar rats (250-300 gms) were sacrificed by decapitation, and the brains or pituitary glands were removed and rinsed in cold Krebs-Ringer buffer and the corpus striatum was dissected. Tissues from 3-4 rats were pooled prior to homogenization in 15 volumes of 50 mM TES, 2 mM EGTA buffer, pH 7.4. A 25 µl aliquot of homogenate (200 µgm protein) was incubated with the following components, shown at final concentrations: 80 mM TES·HCl buffer, pH 7.4; 2 mM $MgSO_4$; 0.5 mM isobutyl-methylxanthine; 20 mM creatine phosphate; 10 units of creatine kinase; 0.2 mM EGTA; and 0.5 mM ^{32}P-α-ATP containing approximately 10^5 cpm/tube. All compounds were prepared immediately before use in 0.1% ascorbic acid. Some antagonists were dissolved in 1 mM HCl. In all cases vehicle controls were included in the same incubations. The reaction was started by adding the freshly prepared homogenate, and samples were

incubated for 3 minutes at 37°C in a shaking water bath. The reaction was terminated by placing the tubes in a boiling water bath for 3 min and then transferring to an ice bath. A 1 ml solution of ^{3}H-cyclic AMP containing about 1500 cpm was then added to each tube and the samples centrifuged for 5 min. at 900 x g. The supernatant was decanted and the pellet discarded.

Purification of cyclic AMP was accomplished by passing the supernatant through a column of neutral alumina which had previously been equilibrated with 0.06 M Tris·HCl, pH 7.4. The effluent from the alumina column drained directly onto a 0.6 x 6 cm column of Dowex 1 (chloride form, washed to neutrality with H_2O). The alumina columns were washed with 5 ml of 0.6 M Tris buffer, pH 7.4 and discarded. The Dowex columns were washed with 5 ml of H_2O and 1 ml of 0.05 N HCl and the effluents discarded. Cyclic AMP was eluted into counting vials with 2 ml 0.05 N HCl and 15 ml of toluene-permafluor-Triton X-100 scintillation solution were added. The samples, along with ^{3}H-cyclic AMP and ^{32}P-ATP standard, were counted for ^{3}H and ^{32}P in a Nuclear Chicago Mark III scintillation counter.

Cyclic AMP synthesis in intact pituitary glands was determined by incubating 1/2 glands in Krebs-Ringer buffer containing 2 mg/ml glucose. Two glands were bisected and incubated in 1 ml buffer for 30 min. at 37°C at which point fresh warmed medium was added and the incubation continued for 15 min. Dopamine solutions were then added, and the reaction was allowed to proceed for 10 min. The medium was removed and 2 ml of 0.3 N perchloric acid containing trace amounts of ^{3}H-cyclic AMP were added to each tube. The samples were transferred to glass homogenizers and homogenized. Aliquots were taken for protein determination (21) and the samples centrifuged for 20 min. at 15,000 x g. The supernatants were neutralized with 2 M Tris base (pH 8.2) and chromatographed over alumina and Dowex 1 as previously described. The cyclic AMP fractions were lyophilized and resuspended in 0.5 ml of NaAC buffer, pH 4.0. Cyclic AMP concentrations were determined by the method of Gilman (22). In both assay systems final values were corrected for loss of cyclic AMP during chromatography and purification.

Wistar rats were purchased from Harlan Industries, Indianapolis, Indiana. Radioactive ATP (7-10 Ci/mmole) was supplied by New England Nuclear, Boston, Mass., and ^{3}H-cyclic AMP (30 Ci/mmole) was from Amersham-Searle. TES buffer (N-tris-(hydroxymethyl) methyl 2-aminoethane sulfonic acid), creatine phosphate, creatine phosphokinase, ATP, propranolol, phentolamine, ergocristine, ergonovine, ergotamine, neutral alumina, Dowex-1 (chloride form, 200-400 mesh) and cyclic AMP were purchased from Sigma Chemical Co. (St. Louis, Mo.). Aldrich Chemical Company supplied 3-iso-butyl-1-methylxanthine. Lergotrile (2-chloro-6-methyl-ergoline-8β-acetonitrile) was synthesized by Dr. E. Kornfeld, Lilly Research Laboratories, Indianapolis, Indiana). Apomorphine hydrochloride was provided by Merck Chemical Company.

Results

The present assay of adenylate cyclase involving purification of end product through alumina and Dowex 1 gives a rapid and accurate

estimate of enzyme activity. Adsorption of excess ^{32}P-ATP by alumina and elution of cyclic AMP from Dowex 1 with HCl reduces blank activity significantly while recovery of ^{32}P-cyclic AMP is greater than 75%. The reaction was linear for at least 10 min. and proportional to homogenate protein concentration from 0-250 µg protein/tube. Dopamine produced a dose-dependent elevation of cyclic AMP synthesis with maximal activity (90-130% increase) seen at 10^{-4}M. The effects of various amine antagonists on dopamine-stimulated enzyme activities are compared in Table 1. The dopamine-induced elevation of cyclic AMP synthesis was inhibited by the dopamine blockers spiroperidol, pimozide and D-butaclamol, but not by low concentrations of an alpha blocker (phentolamine), a beta blocker (propranolol) or the L-form of butaclamol. Dopamine was significantly more active than norepinephrine or epinephrine in increasing activity in striatal homogenates and dopamine was inactive in cerebellar homogenates (data not shown). These results defining the basic parameters of the present adenylate cyclase assay are consistent with the literature with respect to the degree of dopamine stimulation of adenylate cyclase in homogenates of the striatum and the effects of various antagonists on dopamine activity in such systems (23,24,25). These confirmatory experiments were conducted to validate the present adenylate cyclase assay system.

TABLE 1

Effect of Antagonists and Ergots on Dopamine-Stimulated Cyclic AMP Synthesis in Homogenates of Striatum

Compound	IC_{50} (µM)	% Effect on Dopamine (10^{-4}M) Stimulation of Adenyalte Cyclase in Striatum		
		10^{-6}M	10^{-5}M	10^{-4}M
Propranolol	--	+ 4.8	+10.6	+ 0.4
Phenoxybenzamine	90.0	+ 9.1± 6.7	+15.4± 4.4	-54.2±12.4
Pimozide	23.0	-16.1	-41.0	---
Spiroperidol	3.3	-22.1	-77.1	---
(+)-Butaclamol	<1.0	-78.9± 5.0	-82.3± 1.8	---
(-)-Butaclamol	--	0.0	+12.0± 2.2	---
LSD	4.0	-13.8± 8.4	-66.2± 7.8	-72.2± 7.1
Lergotrile	4.2	-30.5± 2.7	-60.3± 3.7	-91.9± 3.9
Ergocristine	12.0	-27.2±11.1	-46.2±11.5	-70.2± 2.7
Ergonovine	19.0	-12.0± 2.3	-43.0± 4.9	-61.8± 3.3
Ergotamine	33.0	-15.9±11.7	-34.1± 6.7	-78.3±16.6

Values represent the means ± S.E.M. of 2-6 determinations. Tissues were prepared and incubated as indicated in the Methods. IC_{50} values were estimated from graphical representations of the data using 10^{-4}M dopamine as stimulant.

All ergots were considerably less active than dopamine as stimulants of striatal adenylate cyclase, (Fig. 1). LSD and ergonovine were most active, ergocristine was intermediate in activity, and lergotrile and ergotamine were essentially inactive. When dopamine and the ergots were added together a significant reduction in the dopamine stimulation of cyclic AMP synthesis was seen, (Table 1). However, neither LSD nor lergotrile (10^{-6}-10^{-4}M)

inhibited the sodium fluoride-activation of adenylate cyclase (data not shown).

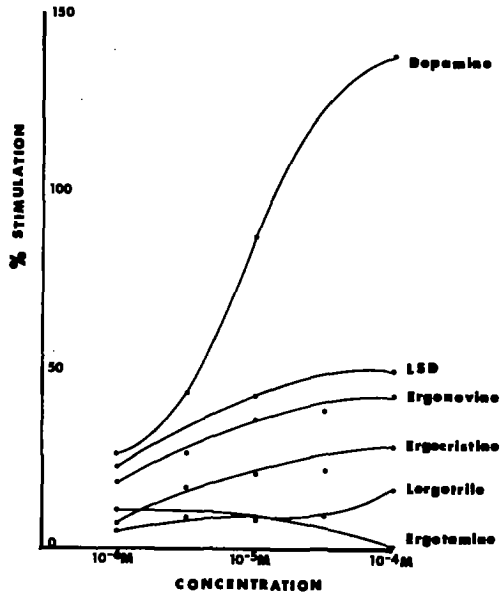

FIG. 1

Effects of Various Ergots on Adenylate Cyclase Activity in Homogenates of the Striatum

Tissues were prepared and incubated as in Methods. Points represent the means from 3 separate experiments with duplicate or triplicate replications within each experiment. Values across experiments differed by less than 5%. Activity in the absence of dopamine was 131.9 pmoles cyclic AMP/mg protein/min. and 308.2 pmoles cyclic AMP/mg protein/min. in the presence of 10^{-4}M dopamine.

Adenylate cyclase activity in homogenates of the pituitary gland was lower than in homogenates of the striatum. A comparison of dopamine and other agents that inhibit the release of prolactin revealed that none was able to stimulate the synthesis of cyclic AMP (Table 2). However, in the same experiment both dopamine and apomorphine significantly increased cyclic AMP synthesis in homogenates of the corpus striatum.

TABLE 2

Comparison of Dopamine Agonists on Adenylate Cyclase in Homogenates of the Pituitary Gland or Striatum

	Pituitary Gland	Corpus Striatum
	pmoles cyclic AMP/mg protein/minute	
Control	66.2± 8.7	152.4± 7.9
Dopamine, 10^{-6}M	67.7± 4.1	168.7± 8.4
10^{-5}M	70.0±10.5	---
10^{-4}M	52.3± 6.3	288.7±17.3
Lergotrile, 10^{-6}M	58.2± 0.5	143.3±19.5
10^{-5}M	58.7± 4.9	---
10^{-4}M	67.0± 7.6	156.7± 9.0
Apomorphine, 10^{-5}M	61.7± 7.8	195.7±12.2

Tissues were prepared and incubated as indicated in the Methods. Values represent the mean ± S.E.M. of three separate tissue preparations with duplicate replications within each assay.

Discussion

It had been reported that LSD could increase cyclic AMP levels in striatal homogenates (20,26) by directly stimulating adenylate cyclase since LSD was not able to inhibit cyclic nucleotide phosphodiesterase (27). Recently Schorderet (14) showed that ergometrine, ergoclavine, but not LSD, increased adenylate cyclase activity of retinal homogenates. These findings supported the contention that the dopamine agonist activity of ergots was mediated by an increase in cyclic AMP concentration in target tissues. We examined a series of structurally diverse ergots in striatal homogenates and found that not all ergot-like compounds stimulate adenylate cyclase (Fig. 1). The most active ergots were LSD and ergonovine which are lysergic acid amides with small alkyl chain substitutions. Ergocristine and ergotamine are also amino acid-type ergots but the substituents on the nitrogen are large bulky groups and these agents were significantly less active than LSD. Lergotrile is not an amino acid-type ergot, possessing only the basic ergoline nucleus, and this compound was inactive as a cyclase stimulant.

The inability of lergotrile to stimulate cyclic AMP synthesis in striatal homogenates was unexpected since *in vivo* the compound behaves like a dopamine agonist. Lergotrile decreases the release of prolactin (28), produces rotational behavior in lesioned rats (J. Clemens, personal communication), reduces dopamine turnover in the brain (29), alleviates tremor activity in tegmental lesioned monkeys and shows beneficial effects in Parkinsonian patients (30). Yet, in no experiment did lergotrile enhance cyclic AMP synthesis. One possible explanation was that lergotrile itself was not the active compound since its effects *in vivo* occur with a delayed onset (29). We examined several potential metabolites of lergotrile and found them to be inactive (data not shown). The fact that lergotrile reduces prolactin release from isolated pituitary glands (28) also indicates that the parent compound has

inherent activity.

Although lergotrile was the most dramatic example of *in vivo* dopaminergic activity not being reflected in the adenylate cyclase assay, incongruities were seen with other ergots. None of the ergots we tested stimulated adenylate cyclase to the degree seen with dopamine (Fig. 1). One reason for this might be the dual agonist-antagonist nature of these compounds. All ergots markedly inhibited the dopamine-induced activation of adenylate cyclase with LSD and lergotrile being most active, (Table 1). This inhibition could be due to the alpha blocking activity of these compounds since phenoxybenzamine at an elevated concentration reduced the dopamine stimulation by 54% (Table 1). Other studies have shown α-adrenergic blockade of dopamine in the striatum (18,24,31), and there are other instances in which ergots exerted biphasic or inhibitory effects on the synthesis of cyclic AMP in the CNS. Palmer and Burks (32) reported that LSD inhibited the norepinephrine-induced elevation of cyclic AMP in slices of brain stem, and Markstein and Wagner (33) found that dihydroergotoxin inhibited cyclic AMP synthesis in cortical slices. Inhibition of dopamine-stimulated synthesis of cyclic AMP has been reported for LSD (20), ergotamine (19) and bromocriptine (34). We have now found this inhibitory activity in several other ergots suggesting that this activity might be predominant in all ergots. The antagonism of dopamine-stimulation appears specific since activation of adenylate cyclase by sodium fluoride was not reduced by LSD or lergotrile (data not shown).

One of the more well-documented effects of ergots is their ability to inhibit the release of prolactin from the pituitary gland. *In vitro* experiments have shown that dopamine (35) and apomorphine (36) can also produce this effect, and specific dopamine receptor binding sites have been found in pituitary membrane fractions (37). We therefore expected dopamine and dopamine agonists to stimulate cyclic AMP synthesis in homogenates of the pituitary gland, but in no experiment was there a detectable increase in cyclic AMP synthesis in the presence of dopamine, apomorphine, or lergotrile, (Table 2). Yet in the same experiment dopamine and apomorphine did stimulate cyclic AMP synthesis in homogenates of the striatum prepared and incubated exactly as the pituitary homogenates. Earlier, Steiner *et al.* (38) reported that epinephrine and norepinephrine did not stimulate adenylate cyclase in pituitary homogenates. Since many tissues lose adenylate cyclase hormone sensitivity when homogenized, we looked for dopamine stimulated cyclic AMP synthesis in intact pituitary glands. However, dopamine did not elevate cyclic AMP concentrations in intact glands even though the release of prolactin was significantly reduced (data not shown).

Recently it has been shown that there is a heterogeneous distribution of dopamine (39), LSD and dopamine-dependent adenylate cyclase (24), and dopamine agonist and antagonist receptors in the striatum (40). Our findings indicate that if dopamine receptors exist in the pituitary gland, as ample *in vivo* and *in vitro* evidence indicates, those dopamine receptors which control prolactin release are not associated with adenylate cyclase, do not function in a manner similar to dopamine receptors in the striatum, or are unlike dopamine receptors in the striatum. Similarly, those dopamine receptors that are activated by

ergolines might be only marginally associated with adenylate cyclase.

Why lergotrile, bromocriptine (34) and other ergots behave as such strong dopamine agonists in in vivo systems and are only marginally active or inactive as adenylate cyclase stimulants in vitro is an enigma and questions the rationale for using the adenylate cyclase assay to assess dopamine agonist activity, at least in the case of ergot-like compounds. However, caution must also be exercised in assuming that an in vivo dopaminergic profile is due to direct dopamine stimulating effects of a compound. One potential problem of in vivo tests is that they often are unable to distinguish direct effects of a compound from nonspecific effects occurring on other receptors or in other distant organ systems which indirectly influence the parameter being monitored. In the case of the ergots this might be especially important since these compounds have been shown to act as alpha blockers (1), beta blockers (41,42), serotonin antagonists (2), serotonin agonists (43), and dopamine antagonists (20), as well as dopamine agonists (44).

References

1. M. NICKERSON, in The Pharmacological Basis of Therapeutics (Goodman, L. S. and Gilman, A. eds.) MacMillan Co., N.Y. pp. 546-577 (1965).
2. G. K. AGHAJANIAN, Ann. Rev. Pharmacology 12 157-168 (1972).
3. D. R. BURT, I. CREESE and S. H. SNYDER, Mol. Pharmacol. 12 631-638 (1976).
4. P. BRAZEAU, in The Pharmacologic Basis of Therapeutics (Goodman, L. S. and Gilman, A. eds.) MacMillan Co., N.Y. pp. 878-886 (1965).
5. D. B. CALNE, P. F. TEYCHENNE, P. N. LEIGH, A. N. BAMJI and A. N. GREENACRE, The Lancet Dec. 7 1355-1356 (1974).
6. T. W. STONE, Brain Res. 72 177-180 (1974).
7. A. J. J. PIJNENBURG, G. N. WOODRUFF and J. M. VAN ROSSUM, Brain Res. 59 289-302 (1973).
8. G. ANLEZORK, C. PYCOCK and B. MELDRUM, Eur. J. Pharmacol. 37 295-302 (1976).
9. A. M. JOHNSON, J. M. VIGORNET, and D. M. LOEW, Experientia 29 763 (1973).
10. I. PODVALOVA and A. DLABAC, Activ. Ner. Sup. (Praha) 12 81 (1970).
11. G. N. WOODRUFF, A. O. ELKHAWAD and A. R. CROSSMAN, J. Pharm. Pharmac. 266 455-456 (1974).
12. T. MIYAMOTO, A. BATTISTA, M. GOLDSTEIN and K. FUXE, J. Pharm. Pharmac. 26 452-454 (1974).
13. H. CORRODI, K. FUXE, T. HOKFELT, P. LIDBRINK and U. UNGERSTEDT, J. Pharm. Pharmac. 25 409-412 (1973).
14. M. SCHORDERET, Neuroscience Letters 2 87-91 (1976).
15. J. A. CLEMENS, C. J. SHAAR, E. B. SMALSTIG, N. J. BACH and E. C. KORNFELD, Endocrinology 94 1171-1176 (1974).
16. K. FUXE, L. F. AGNATI, H. CORRODI, B. J. EVERITT, T. HOKFELT, A. LOFSTROM and U. UNGERSTEDT, Adv. Neurology 9 223-239 (1975).
17. H. SATOH, Y. SATOH, Y. NOTSU, and F. HONDA, Jap. J. Pharmacol. 25 Supp. 63p, (1975).
18. J. W. KEBABIAN, G. L. PETZOLD and P. GREENGARD, Proc. Nat. Acad. Sci. 69 2145-2149 (1972).
19. L. L. IVERSEN, Science 188 1084-1089 (1975).

20. K. VON HUNGEN, S. ROBERTS and D. F. HILL, Nature 252 586-588 (1974).
21. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. Biol. Chem. 193 265-275 (1951).
22. A. G. GILMAN, Proc. Nat. Acad. Sci. 67 305-312 (1970).
23. R. MILLER, A. HORN and L. IVERSON, Nature 250 238-241 (1974).
24. J. BOCKART, J. PREMONT, J. GLOWINSKI, A. M. THIERRY and J. P. TASSIN, Brain Res. 107 303-315 (1976).
25. Y. C. CLEMENT-CORMIER, J. W. KEBABIAN, G. L. PETZOLD and P. GREENGARD, Proc. Nat. Acad. Sci. 71 1113-1117 (1974).
26. M. DA PRADA, A. SANER, W. P. BURKHARD, G. BARTHOLINI and A. PLETSCHER, Brain Res. 94 67-73 (1975).
27. P. UZUNOV and B. WEISS, in Advances in Cyclic Nucleotide Research, (Greengard, P. and Robison, G. A., eds.) Raven Press, N.Y., Vol. 1, pp. 435-453 (1972).
28. J. A. CLEMENS, E. B. SMALSTIG and C. J. SHAAR, Acta Endocrinologia 7 230-237 (1975).
29. K. W. PERRY and R. W. FULLER, Abstract, Sixth Annual Meeting of the Society for Neuroscience, (1976).
30. A. LIEBERMAN, T. MIYAMOTO, A. BATTISTA, M. GOLDSTEIN, Neurology 25 459-462 (1975).
31. N.-E. ANDEN, A. DAHLSTROM, K. FUXE and K. LARSSON, Acta Pharmacol. Toxicol. 24 263-274 (1966).
32. G. C. PALMER and T. BURKS, Eur. J. Pharmacol. 16 113-116 (1971).
33. R. MARKSTEIN and H. WAGNER, FEBS Letters 55 275-277 (1975).
34. M. TRABUCCHI, P. F. SPANO, G. C. TONON and L. FRATTOLA, Life Sci. 19 225-232 (1976).
35. C. J. SHAAR and J. A. CLEMENS, Endocrinology 95 1202-1212 (1974).
36. E. B. SMALSTIG, B. D. SAWYER and J. A. CLEMENS, Endocrinology 5 123-129 (1974).
37. G. M. BROWN, P. SEEMAN, T. LEE, Endocrinology 99 1407-1410 (1976).
38. A. L. STEINER, G. T. PEAKE, R. D. UTIGER, I. E. KARL and D. M. KIPNIS, Endocrinology 86 1345-1360 (1970).
39. J. P. TASSIN, A. CHERAMY, G. BLANC, A. M. THIERRY and J. GLOWINSKI, Brain Res. 107 291-301 (1976).
40. A. R. COOLS, H. A. J. STRUYKER BOUDIER and J. M. VAN ROSSUM, Eur. J. Pharmacol. 37 283-293 (1976).
41. P. S. CHAN and L. ELLENBOGEN, Arch. int. Pharmacodyn. 209 204-213 (1974).
42. R. MAURER and A. GRIEDER, Experientia 31 730-731 (1976).
43. H. CORRODI, Eur. J. Pharmacol. 30 172-181 (1975).
44. K. FUXE, L. AGNATI, T. HOKFELT, G. JONSSON, P. LIDBRINK, A. LJUNGDAHL, A. LAPTROM, and U. UNGERSTEDT, J. Pharmacol. (Paris) 6 117-129 (1975).