Arch. Psychiatr. Nervenkr. 220, 325—333 (1975)

Cyclic Adenosinemonophosphate in Cerebrospinal Fluid

Effects of Theophylline, L-Dopa and a Dopamine Receptor Stimulant in Rats *

Marika Kießling, Toni Lindl and Hinrich Cramer

Neurologische Universitätsklinik (Prof. R. Jung) Freiburg i. Br.

Received June 28, 1975

Summary. The effects of L-Dopa and the dopamine receptor stimulant ET-495 on cisternal cAMP levels were studied in rats. L-Dopa (100—200 mg/kg) increased cisternal cAMP levels by 60 to 80% of controls. When peripheral Dopa-decarboxylase was inhibited, smaller doses of L-Dopa were effective. Fla-63, an inhibitor of dopamine-β-hydroxylase lowered the increase induced by L-Dopa which was completely suppressed by propranolol, not by phentolamine, suggesting that the cAMP increase is mediated through a central β-adrenoceptor stimulation. ET-495 failed to influence cAMP levels which argues against a dopamine-sensitive adenylate cyclase involved in the L-Dopa effect. Moreover, large increases of cisternal cAMP were observed after treatment with theophylline, not papaverine which suggests different effects of these "phosphodiesterase inhibitors" on the cyclic AMP systems in the central nervous system or on transport mechanisms.

Key words: Adenosine 3′,5′-Monophosphate — Cerebrospinal Fluid — L-Dopa — Catecholamines — Theophylline — Piribedil.

Zusammenfassung. Die Wirkung von L-Dopa und dem Dopaminagonisten ET-495 auf die cisternale cAMP-Konzentration wurde bei Ratten untersucht. L-Dopa (100—200 mg/kg) erhöhte die cAMP-Spiegel um 60—80%. Den gleichen Effekt hatten geringere L-Dopa-Dosen nach Hemmung der extracerebralen Dopa-Dekarboxylase. Vorbehandlung mit dem Dopamin-β-Hydroxylase-Hemmer Fla-63 verminderte die Wirkung von L-Dopa, welche durch Propranolol völlig unterdrückt wurde. ET-495 beeinflußte die cisternale cAMP-Konzentration nicht. Es wird geschlossen, daß der cAMP-Anstieg nach L-Dopa-Anwendung über einen zentralen noradrenergen Mechanismus mit Stimulierung eines β-adrenergen Adenylatcyklase-Receptors zustande kommt. Es wird ferner gezeigt, daß Theophyllin, nicht aber Papaverin, die cAMP-Konzentration im Liquor stark erhöht, was unterschiedliche Wirkungen der beiden „Phosphodiesterasehemmer" auf den Stoffwechsel oder Transport von cAMP nahelegt.

Schlüsselwörter: Cyclisches AMP — Liquor cerebrospinalis — L-Dopa — Catecholamine — Theophyllin — Dopaminreceptorstimulans — Piribedil.

Introduction

Postsynaptic effects of several putative neurotransmitters appear to be mediated by adenosine 3′,5′-monophosphate (cAMP) as a "second messenger" (Rall, 1972). Noradrenaline and adrenaline rapidly increase cAMP levels in peripheral and central nervous tissues when applied *in vitro* or *in vivo* (Cramer *et al.*, 1973; Westermann, 1973). Dopamine is capable of activating adenylate cyclase in brain homogenates (Kebabian *et al.*, 1972) but unable to increase cAMP levels in intact brain tissue (Kakiuchi and Rall, 1968). In peripheral sympathetic ganglia of rabbits dopamine increased cAMP concentrations when used together

* Supported by the Deutsche Forschungsgemeinschaft (SFB 70). Work presented in this paper is part of a thesis to be submitted to the Medical Faculty of the University of Freiburg (M. K.).

with theophylline (Kalix *et al.*, 1974) but not in rat ganglia with or without theophylline (Cramer *et al.*, 1973; Lindl and Cramer, 1975). Controversy thus exists as to the existance of a dopamine-sensitive adenylate cyclase in the intact brain. It has been hypothesized that cAMP is involved in dopaminergic transmission and that derangements of cAMP metabolism might be involved in the defect leading to Parkinson's disease (Kebabian *et al.*, 1972). However, in parkinsonian patients no changes in cerebrospinal fluid (CSF) cAMP could be found, and L-Dopa treatment did not alter lumbar cAMP levels in these patients or in control subjects (Cramer *et al.*, 1973b). Since L-Dopa is a precursor for both dopamine and noradrenaline and readily crosses the blood brain barrier after systemic administration—allowing its therapeutic use in disorders of central catecholamine metabolism—possible influences of the drug on cAMP systems remain of paramount interest.

Despite the demonstration of potent cAMP generating systems, high tissue levels of cAMP and rapid responses to activation of specific receptors for adenylate cyclase, the significance of cAMP systems in neurotransmission processes and brain metabolism remains far from clear. Pathophysiologic aspects and therapeutic avenues are largely unexplored. One approach to study cerebral matabolism and drug effects both in animals and in humans is to follow the levels of metabolites in the cerebrospinal fluid (CSF), an approach which has been followed successfully in the study of biogenic amines (Gerbode and Bowers, 1968) and proposed for cyclic nucleotides (Cramer *et al.*, 1972). The aim of these experiments is to further substantiate the possibility to study central cyclic nucleotide metabolism by monitoring CSF changes in experimental as well as in clinical conditions. One of the most successful drugs in the treatment of Parkinson's disease is L-Dopa. Therefore experiments were designed in rats in order to measure and evaluate possible changes in CSF levels of cAMP after the administration of L-Dopa and drugs mimicking its therapeutic actions or interfering with its effects.

Materials and Methods

For the experiments male SIF-50 rats of 250 to 350 g body weight were used. The animals were fed ad libitum with Altromin chow for rodents and with water. Cisternal CSF was obtained by suboccipital puncture with a gauge 2 cannula under light ether anesthesia. The duration of exposure to ether was 2 to 3 min before CSF was obtained. From each animal 0.05 to 0.12 ml CSF was subtracted; each animal was used only once. Only clear CSF was used for further analysis. cAMP was determined in CSF according to Gilman (1970).

Drugs were freshly dissolved and applied intraperitoneally, control animals received an equivalent amount of solvent only. L-Dopa was obtained from Ciba-Geigy, Basle, Ro 4-4602 (N-(DL-seryl)-N′-2,3,4-trihydroxy-benzyl)-hydrazine (HCl)) from Hoffmann-LaRoche, Basle, (±)-propranolol-HCl from Rhein-Pharma, Heidelberg, Phentolamine-HCl from Ciba-Geigy, ET-495 (Piribedil) from Servier S.A., Neuilly, and Fla-63 (bis-(4-methyl-1-homopiperazinyl thiocarbonyl)-disulfide) from Astra Ltd., Södertälje. All other chemicals used were reagent grade obtained from commercial sources.

Results

To establish whether changes of cisternal cAMP levels parallel changes of cerebral cAMP levels, drugs known to influence the latter were applied to rats. Theophyline has been widely used to inhibit cerebral cAMP phosphodiesterase and is a drug capable of increasing baseline and drug-induced levels of cAMP in

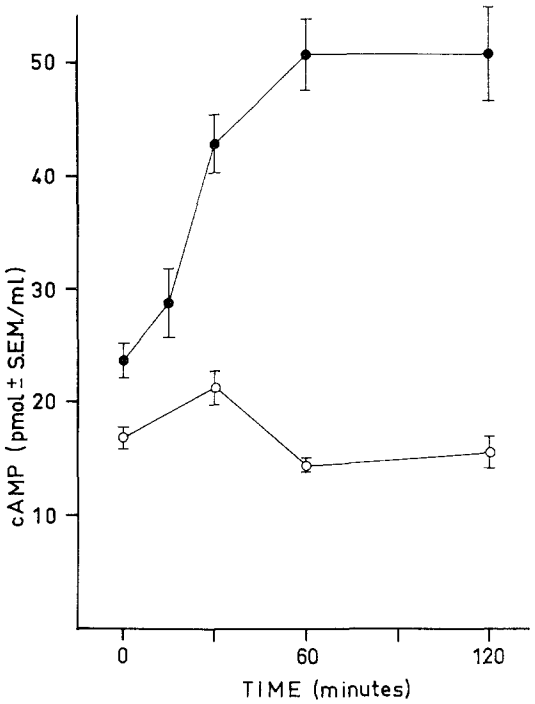

Fig. 1. Time course of the increase of cAMP levels in CSF after i.p. administration of theophylline (80 mg/kg, filled circles) and papaverine (80 mg/kg, open circles). The mean levels are expressed as pmoles cAMP ± S.E.M./ml CSF

brain slices (Kakiuchi and Rall, 1968). A nonconvulsant dose of theophylline (80 mg/kg i.p.) increased cisternal cAMP levels more than two fold after 60 min (Fig. 1). Theophylline inhibits phosphodiesterase instantaneously and apparently the increase of cisternal cAMP follows intercerebral accumulation with only a small lag time. Papaverine which also strongly inhibits cAMP phosphodiesterase in several tissues (Kukovetz and Pöch, 1970) failed to increase the CSF cAMP levels when used at equimolar dosage (Fig. 1).

L-Dopa (200 mg/kg) increased cAMP levels with an intial time course similar to that of theophylline but levels declined again after 45 min (Fig. 2). It is well known that L-Dopa is rapidly taken up into brain cells and converted to catecholamines. A dose response curve for L-Dopa showed maximal effects at a dose of 100 mg/kg with no further increase at higher doses (Fig. 3). 100 mg/kg probably is sufficient to saturate uptake in catecholamine neurones, at very high doses uptake in other neurons with displacement of serotonin has been shown (Everett and Borcherding, 1970). When L-Dopa was applied together with a peripheral decarboxylase inhibitor (Bartholini *et al.*, 1967) much lower doses of L-Dopa produced similar increases (Fig. 2), excluding that the cisternal cAMP is derived from peripheral sources or brain capillaries.

L-Dopa increases both dopamine and noradrenaline in brain and indirectly may activate adenylate cyclase via dopamine and/or noradrenaline receptors.

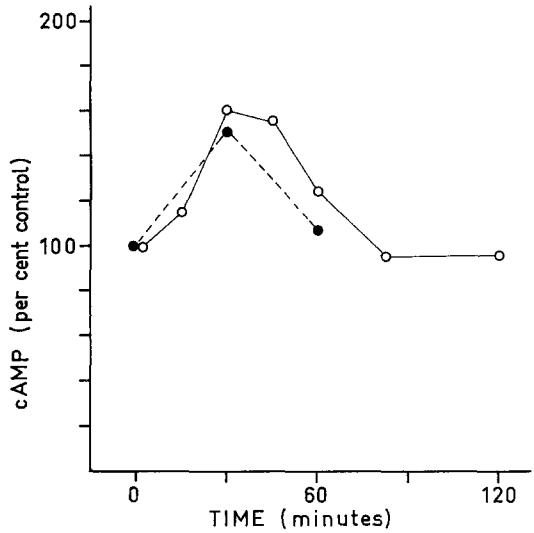

Fig. 2. Time course of the increase of cAMP levels in CSF after i.p. administration of L-Dopa (200 mg/kg, open circles), and L-Dopa (40 mg/kg) given 10 min after Ro-4602 (50 mg/kg) (filled circles). The data are expressed as percent of controls

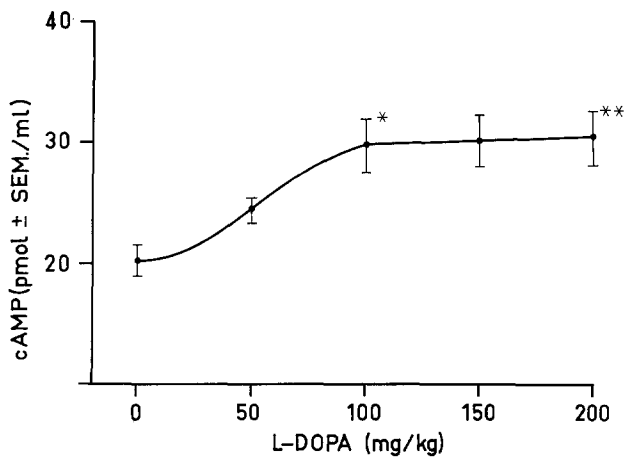

Fig. 3. Dose response curve for the effect of L-Dopa on cAMP levels in cisternal CSF, obtained 30 min after the injection of L-Dopa. *: $P < 0.05$ for difference from control; **: $P < 0.001$ for difference from control (Student's t-test)

Influences on the metabolism of both catecholamines are thought to be important in the therapeutic action of the drug, because drugs acting predominantly on dopaminergic receptors (such as apomorphine and ET-495) are considerably weaker than L-Dopa as antiparkinson agents (Hornykiewicz, 1974). To clarify whether cAMP after application of L-Dopa is increased in CSF by activation of a dopamine of a noradrenaline sensitive cyclase the effects of an inhibitor of dopamine-β-hydroxylase (FLA-63, Florvall and Corrodi, 1970) and of a pure

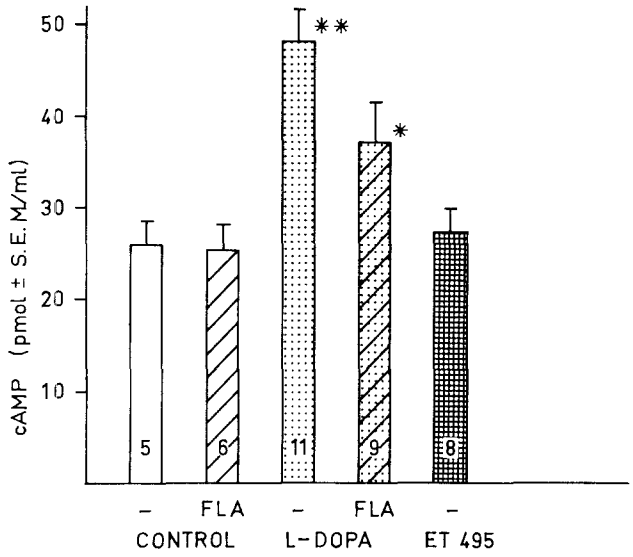

Fig. 4. Effect of Fla-63 (FLA) on the L-Dopa induced increase, and of ET-495 on CSF levels of cAMP. Fla-63 (50 mg/kg) was given 60 min prior to the injection of L-Dopa (200 mg/kg). CSF was obtained 30 min after the administration of L-Dopa or ET-495 (10 mg/kg). The numbers in columns show the number of rats for each group. * and **: $P < 0.05$ and $P < 0.001$ for difference from control

dopamine receptor stimulating agent (ET-495) were studied. FLA-63 markedly diminuished the cAMP response to L-Dopa (Fig. 4) but it did not suppress it. The inhibition of dopamine-β-hydroxylase by a well tolerated dose of FLA-63 can hardly be expected to be complete *in vivo* and when substrate is increased maximally by L-Dopa. ET-495, a potent dopamine receptor stimulant in the central nervous system (Corrodi *et al.*, 1971) on the other hand failed completely to influence cisternal cAMP levels (Fig. 4).

Since from the foregoing experiments a noradrenaline sensitive adenylate cyclase appears to be activated after the administration of L-Dopa, we tried to further characterize the response by pharmacologic interferences. Propranolol which is a selective β-adrenergic blocking compound and suppresses cAMP accumulation induced by noradrenaline in a number of nervous tissue preparations, completely suppressed the accumulation of cAMP in rat cisternal fluid after L-Dopa (Fig. 5). Propranolol alone did neither increase nor decrease cAMP levels. Phentolamine, a compound which blocks α-adrenoceptors failed to diminuish the response to L-Dopa (Fig. 5). Surprisingly, phentolamine alone (2.5 or 5 mg/kg) was capable of increasing cAMP levels, although slightly less than L-Dopa (Fig. 5). While some Eigenwirkung of this blocker on adrenergic receptors is conceivable and phentolamine may release catecholamines (Goodman and Gilman, 1970), their is an intriguing possibility that the drug eliminates an α-adrenergic presynaptic feedback mechanism which normally limits noradrenaline release at synapses (Starke, 1973). The combined administration of phentolamine and propranolol suppressed the accumulation of cAMP in CSF (Fig. 5).

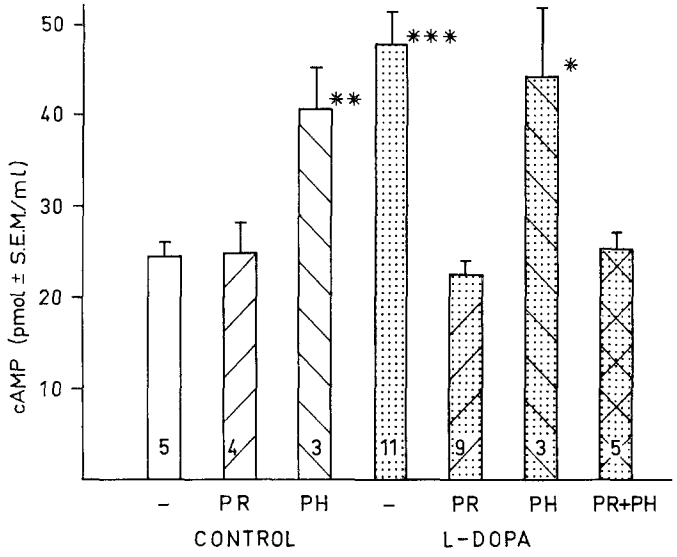

Fig. 5. Effect of propranolol (10 mg/kg, PR) and phentolamine (5 mg/kg, PH) on CSF levels of cAMP and on the L-Dopa induced accumulation of cAMP. The blockers were given 10 min prior to L-Dopa, i.e. 40 min before the subtraction of CSF. *: $P < 0.05$; **: $P < 0.02$; ***: $P < 0.001$ for differences from control (no drug)

Discussion

Previously it has been shown that CSF concentrations of cAMP may vary independently of blood levels and that cAMP in CSF is probably derived from the central nervous system (Cramer *et al.*, 1972; Sebens and Korf, 1975).

The present results further indicate that studies of CSF cAMP are valuable for the evaluation of the activity of cAMP-generating systems within the brain in different conditions *in vivo*. Intracellular metabolite concentrations are generally subject to mechanisms which maintain or restore steady state conditions. Besides cAMP-hydrolyzing enzymes, release or overflow of cyclic nucleotides into blood and CSF may form an important part of means of nucleotide removal (Cramer and Lindl, 1974). This is also supported by the marked increase in CSF cAMP after inhibition of cAMP phosphodiesterase by theophylline reported in this study. However, theophylline possibly has other central effects than actions on phosphodiesterases and there is no proof that the increase in cAMP is effected through inhibition of these enzymes. Interestingly, papaverine, which in a number of tissues acts as a potent phosphodiesterase inhibitor but which also is known to have central effects quite different from theophylline, failed to increase cisternal cAMP levels in these experiments.

Similar to findings of CSF increases in homovanillic acid and 5-hydroxy-indoleacetic acid as indicators of presynaptic metabolic events after L-Dopa administration (Gerbode and Bowers, 1968), changes of cAMP concentrations may serve to evaluate postsynaptic metabolic effects in the second messenger system.

While in man neither chronic treatment nor acute infusion of L-Dopa was able to increase lumbar cAMP concentrations (Cramer *et al.*, 1973b), a clearcut accumulation of cAMP is seen in cisternal fluid in rats. The increase in CSF cAMP after L-Dopa is moderate and transient as compared to the larger and longlasting effect of theophylline which is in agreement with the rapid and short-lasting increase in catecholamine synthesis produced by L-Dopa in rats (Everett and Borcherding, 1970). It is conceivable that lumbar CSF levels in man may reflect only drastic and/or sustained cerebral metabolite changes while small and transient changes are without influence. This is also supported by the lack of spontaneous circadian variations in human lumber fluid cAMP, in contrast to marked oscillations of cAMP levels in ventricular fluid of patients (Cramer *et al.*, 1975).

In experimental animals it has recently been shown that L-Dopa enhances cAMP levels in the caudate nucleus but not in cerebellum (Garelis and Neff, 1974). This effect was blocked when cerebral Dopa-decarboxylase was inhibited by very high doses of Ro 4-4602.

In contrast to L-Dopa, ET-495 (Piribedil) which stimulates central dopamine receptors (Corrodi *et al.*, 1971), failed to increase cisternal cAMP. FLA-63 which inhibits the enzymic conversion of dopamine to noradrenaline (Florvall and Corrodi, 1970) partially inhibited the rise observed after L-Dopa application. Recently, intracisternally injected noradrenaline has been reported to increase cisternal cAMP levels in rabbits, while dopamine had only weak and delayed effects (Sebens and Korf, 1975) which suggests indirect actions. In that study nonconclusive results were obtained with L-Dopa and therapeutic drugs acting on central catecholaminergic mechanisms. In brain slices (Kakiuchi and Rall, 1968) and peripheral sympathetic ganglia of rats (Cramer *et al.*, 1973) dopamine, in contrast to noradrenaline and adrenaline, failled to increase cAMP levels. The existance of a "dopamine-sensitive adenylate cyclase" in the intact animal was concluded from the ability of dopamine to activate adenylate cyclase in brain homogenates (Kebabian *et al.*, 1972) but receptor-enzyme relationships and stereospecificity of transmitter-receptor interactions may be gravely impaired in such preparations. While in homogenates of rat caudate 40 μM dopamine caused a maximal increase in cAMP formation of about 100% over baseline values (Kebabian *et al.*, 1972), in caudate slices half maximal activation of the enzyme was achieved by a concentration of 60 μM dopamine, as compared to 30 μM noradrenaline and 0.03 μM for isoprenaline as a pure β-adrenergic stimulator (Forn *et al.*, 1974). Dopamine-sensitive adenylate cyclase in brain homogenates is inhibited by various neuroleptic and tricyclic antidepressant compounds. However, no correlation was apparent between the capacity of the drugs to inhibit the enzyme, and their antipsychotic potency (Karobath, 1975).

Our experiments do not support the existance of a dopamine-sensitive adenylate cyclase in rat brain *in vivo*. Since dopamine containing structures such as the striatum and the median eminence line the ventricular cavities and increased dopaminergic activity is readily reflected by increased dopamine metabolite concentrations in CSF down to the lumbar level (Gerbode and Bower, 1968), a marked accumulation of cAMP in those structures should not go unobserved in the cisternal fluid.

The finding that L-Dopa increases cAMP by a noradrenergic mechanism certainly does not exclude a functional role of the nucleotide in the extrapyramidal system or in the therapeutic effects of the amino acid. Other evidence suggests that noradrenaline formed from L-Dopa substantially contributes to its effectiveness as an anti-akinesia drug (Hornykiewicz, 1974). In our experiments propranolol, a β-adrenergic blocking agent, completely suppressed the accumulation of cAMP in cisternal fluid. β-adrenergic blocking agents also suppress cAMP accumulation by noradrenaline or adrenaline in many cerebral tissues *in vitro* (Kakiuchi and Rall, 1968) and by isoproterenol *in vivo* (Westermann, 1973). On the other hand dopamine effects in nervous tissues are generally blocked by α-adrenergic blockers such as phentolamine (Kalix *et al.*, 1974; Kebabian *et al.*, 1972). Phentolamine was unable to inhibit the L-Dopa induced accumulation of cAMP in our experiments but phentolamine alone, in contrast to propranolol, was capable of increasing cAMP significantly. Phentolamine, in addition to act as a blocker of α-adrenoceptors, is known to have important "sympathomimetic" actions (Goodman and Gilman, 1970). One may speculate that these actions on pre- or postsynaptic catecholaminergic structures in brain are responsible for the increase in cisternal cAMP.

References

Bartholini, G., Burkard, W. P., Pletscher, A., Bates, H. M.: Increase of cerebral catecholamines caused by 3,4-Dopa after inhibition of peripheral decarboxylase. Nature (Lond.) **215**, 852—853 (1967)

Corrodi, H., Fuxe, K., Ungerstedt, U.: Evidence for a new type of dopamine receptor stimulating agent. J. Pharm. Pharmacol. **23**, 989—991 (1971)

Cramer, H., Johnson, D. G., Hanbauer, I., Silberstein, S. D., Kopin, I. J.: Accumulation of adenosine 3′,5′-monophosphate induced by catecholamines in the rat superior cervical ganglion *in vitro*. Brain Res. **53**, 97—104 (1973)

Cramer, H., Lindl, T.: Release of cyclic AMP from rat superior cervical ganglia after stimulation of synthesis *in vitro*. Nature (Lond.) **249**, 380—382 (1974)

Cramer, N., Ng, L. K. Y., Chase, T. N.: Cyclic AMP: Probenecid-induced rise in human cerebrospinal fluid. J. Neurochem. **19**, 1601—1602 (1972)

Cramer, N., Ng, L. K. Y., Chase, T. N.: Adenosine 3′,5′-monophosphate in cerebrospinal fluid. Effect of drugs and neurologic disease. Arch. Neurol. (Chic.) **29**, 197—199 (1973b)

Cramer, H., Renaud, B., Ortega-Suhrkamp, E.: Konzentration von cyclo-AMP im lumbalen, zisternalen und ventrikulären Liquor bei neurologischen Patienten. Z. Klin. Chem. klin. Biochem. **13**, 245 (1975)

Everett, G. M., Borcherding, J. W.: L-Dopa: Effect on concentrations of dopamine, norepinephrine and serotonin in brains of mice. Science **168**, 849—850 (1970)

Florvall, L., Corrodi, H.: Dopamine β-hydroxylase inhibitors. Acta pharm. svecia **7**, 7—22 (1970)

Forn, J., Krueger, B. K., Greengard, P.: Adenosine 3′,5′-monophosphate content in rat caudate nucleus: demonstration of dopaminergic and adrenergic receptors. Science **186**, 1118—1119 (1975)

Garelis, E., Neff, N. H.: Cyclic Adenosine monophosphate: selective increase in caudate after administration of L-Dopa. Science **183**, 532—533 (1974)

Gerbode, F. A., Bowers, M. B.: Measurement of acid monoamine metabolites in human and animal cerebrospinal fluid. J. Neurochem. **15**, 1053—1055 (1968)

Gilman, A. G.: A protein binding assay for adenosine 3′,5′-monophosphate. Proc. nat. Acad. Sci. (Wash.) **67**, 305—312 (1970)

Goodman, L. S., Gilman, A.: The Pharmacological Basis of Therapeutics. Fourth ed., p. 560. New York-London-Toronto: Macmillan 1970

Hornykiewicz, O.: The mechanisms of action of L-Dopa in Parkinson's disease. Life Sci. **15**, 1249–1259 (1974)

Kakiuchi, S., Rall, T. W.: Studies on adenosine 3′,5′-phosphate in rabbit cerebral cortex. Molec. Pharmacol. **4**, 379–388 (1968)

Kalix, P., McAfee, D. A., Schorderet, M., Greengard, P.: Pharmacological analysis of synaptically mediated increase in cyclic adenosine monophosphate in rabbit superior cervical ganglion. J. Pharmacol. exp. Ther. **188**, 676–687 (1974)

Karobath, M. E.: Tricyclic antidepressive drugs and dopamine-sensitive adenylate cyclase from rat brain striatum. Europ. J. Pharmacol. **30**, 159–163 (1975)

Kebabian, J. W., Petzold, G. L., Greengard, P.: Dopamine sensitive adenylate cyclase in the caudate of rat brain and its similarity to the "dopamine receptor". Proc. nat. Acad. Sci. (Wash.) **69**, 2145–2149 (1972)

Kukovetz, W. R. N., Pöch, G.: Inhibition of cyclic-3′,5′-nucleotide-phosphodiesterase as a possible mode of action of papaverine and similar acting drugs. Naunyn-Schmiedebergs Arch. Pharmak. **267**, 189–194 (1970)

Lindl, T., Cramer, H.: Evidence against dopamine as the mediator of the rise of cyclic AMP in the superior cervical ganglion of the rat. Biochem. Biophys. Res. Commun. **65**, 731–739 (1975)

Rall, T. W.: Role of adenosine 3′,5′-monophosphate (cyclic AMP) in actions of catecholamines. Pharmacol. Rev. **24**, 399–409 (1972)

Sebens, J. B., Korf, J.: Cyclic AMP in cerebrospinal fluid: Accumulation following probenecid and biogenic amines. Exp. Neurol. **46**, 333–344 (1974)

Starke, K.: Regulation of catecholamine release: α-receptor mediated feed-back control in peripheral and central neurones. In: Frontiers in Catecholamine Research, E. Usdin and S. Snyder, eds. pp. 561–565. New York-Toronto-Oxford-Sydney-Braunschweig: Pergamon Press 1973

Strömberg, U., Svensson, T. H.: L-Dopa induced effects on motor activity in mice after inhibition of dopamine-β-hydroxylase. Psychopharmacologia (Berl.) **19**, 53–60 (1971)

Westermann, E.: Effects of catecholamines and prostaglandins on cyclic AMP levels in brain *in vivo*. In: Frontiers in Catecholamine Research, E. Usdin and S. Snyder, eds., pp. 339–343. New York-Toronto-Oxford-Sydney-Braunschweig: Pergamon Press 1973

Prof. Hinrich Cramer
Neurologische Klinik
D-7800 Freiburg i. Br.
Hansastraße 9
Federal Republic of Germany