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The Effects of Probenecid on Cyclic Adenosine 3′, 5′-Monophosphate Levels in Cerebrospinal Fluid and on Brain Phosphodiesterase Activity in the Rat

R. Horstmann*, R. Hammers, P. Clarenbach, and H. Cramer*

Department of Clinical Neurology and Neurophysiology, University of Freiburg, Hansastr. 9, D-7800 Freiburg, Federal Republic of Germany

Summary. In rats, probenecid exhibits a dose-dependent increase in the concentration of cyclic adenosine 3′, 5′-monophosphate (cAMP) in cisternal cerebrospinal fluid (CSF). Maximal accumulation is reached 2 h after IP administration at a dosage of 150 mg/kg body weight. Serum levels of cAMP are unchanged after 200 mg/kg probenecid. In vitro investigations show an inhibitory effect of probenecid on the uptake of cAMP into the isolated choroid plexus of the rabbit. A non-competetive inhibition of probenecid on a high affinity fraction of cyclic nucleotide phosphodiesterase from rat brain homogenates is demonstrated with an inhibitor constant of 3.4×10^{-3}M. The results appear to validitate the "probenecid test" for cAMP in clinical diagnostics.

Key words: Adenosine 3′, 5′-monophosphate – Probenecid – Cerebrospinal fluid – Choroid plexus – Transport – Phosphodiesterase – Brain

Zusammenfassung. Probenecid bewirkt bei Ratten einen dosisabhängigen Anstieg der Konzentration von zyklischem Adenosin-3′, 5′-Monophosphat (cAMP) im zisternalen Liquor. Maximaler Anstieg durch Sättigung des Anionencarriers erfolgt 2 h nach einer Dosis von 150 mg/kg Körpergewicht. Am isolierten Plexus chorioideus läßt sich eine überwiegend kompetitive Hemmung der Aufnahme von cAMP nachweisen. Probenecid hemmt ferner nichtkompetitiv eine für cAMP hochaffine Phosphodiesterase des Rattenhirns mit einer Inhibitorkonstante von 3.4×10^{-3}M. Nachdem Probenecid als Testsubstanz in der neurologisch-psychiatrischen Diagnostik für die Untersuchung des Dopamin- und Serotonin-Umsatzes im Liquor bereits vielfach eingesetzt wird, stützen diese Befunde seine Verwendbarkeit auch für cAMP als second messenger.

* Present address: Scripps Clinic and Institute, La Jolla, CA (USA)
Offprint requests to H. Cramer at the above address

Introduction

The determination of brain metabolites in cerebrospinal fluid (CSF) is one of the most promising approaches applying basic knowledge for diagnostic purposes (Moir et al. 1970). This approach has been used for the biogenic amines dopamine and serotonin which are established transmitter substances in the brain. The CSF concentrations of their metabolites homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) respectively, correlate to a certain degree with their central synthesis rate (Gottfries et al. 1969; Bowers 1970).

The value of this method was improved by the introduction of probenecid, a drug known to inhibit a renal carrier for weak organic anions (Boger et al. 1950). A similar anion transport system capable of eliminating from the CSF such brain metabolites as HVA and 5-HIAA (Guldberg et al. 1966; Neff et al. 1964; Forn 1972) was shown to exist in the choroid plexus and other parts of the ventricular and subarachnoid space lining. Following inhibition of this transport system by probenecid, the accumulation of HVA and 5-HIAA in CSF seems to be a better reflection of the brain metabolism of their precursors than do their native concentrations (van Praag et al. 1970; Chase and Ng 1970; Bowers and van Woert 1972). In humans and in experimental animals an acummulation of cyclic adenosine 3′, 5′-monophosphate (cAMP) in the CSF has also been observed following probenecid treatment (Cramer et al. 1972; Cramer and Lindl 1972; Sebens and Korf 1974). Together with the in vitro demonstration of a probenecid sensitive uptake of cyclic nucleotides into the choroid plexus (Hammers et al. 1977), these results suggest the usefulness of the "probenecid test" for cAMP in neurological diagnostics. The experiments presented in this paper were performed in order to obtain more basic pharmacological data concerning the effects of probenecid on transport and metabolism of cAMP.

Materials and Methods

For in vivo investigations and for cerebral phosphodiesterase studies, male SIF-50 rats of 200 to 350 g body weight were used. The animals were fed with Altromin chow for rodents with water ad lib.

Cisternal CSF was obtained by suboccipital puncture with a gauge 2 cannula under light ether anaesthesia. The exposure to ether started 2 to 3 min before puncture. A volume of 0.05 to 0.12 ml CSF was aspirated, and only clear samples were used for further analysis. Blood was obtained from the carotid arteries following decapitation and samples immediately centrifuged at 1200 g at 0° C, deproteinized with four volumes of ethanol and recentrifuged. The supernatants were evaporated at 50° C and dissowed in 50 mM acetate buffer pH 4.0.

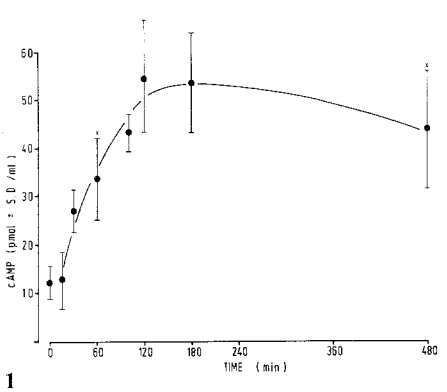
cAMP was determined by protein binding assay according to Gilman (1970). For determinations of cerebral phosphodiesterase activity, rats were killed by cervical dislocation. Brain homogenates were prepared according to Chasin and Harris (1972). Phosphodiesterase activity was assayed using the method of Thompson and Appleman (1971). Probenecid was obtained from Merck, Sharp and Dohme, West Point, Pa, USA. All other chemicals used were reagent grade obtained from commercial sources.

Results

In *untreated animals* the concentration of cAMP in cisternal CSF was 13.1 ± 6.34 pmol/ml. When CSF was obtained 15 min to 6 h after IP injection of 1 ml isotonic

saline, the mean value of the cAMP concentrations was 12.17 ± 3.17 pmol/ml. There was no significant difference between these two groups as to when during the day or at what time following the injection the CSF samples were taken.

A several fold increase in cisternal cAMP concentration was observed after IP *probenecid* (200 mg/kg body weight). A maximum accumulation of cAMP was reached 2 h after the injection followed by a slight but insignificant decrease 6 h later (Fig. 1).



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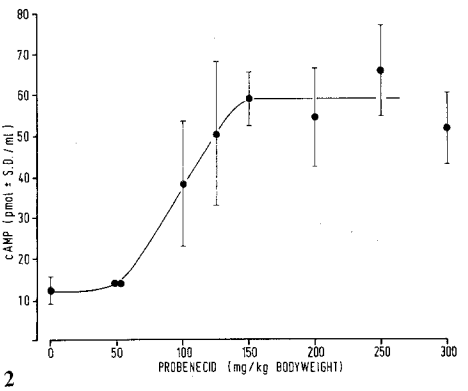

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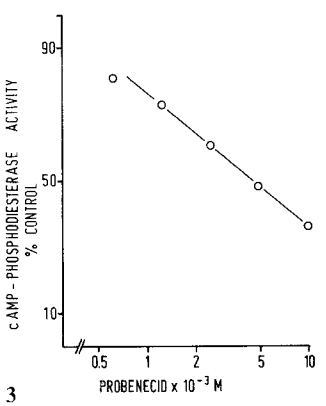
Fig. 1. Time course of cAMP concentrations in cisternal CSF of the rat after IP injection of probenecid (200 mg/kg body weight $n=4$ to 8 rats). The increase after 60 min is significant, $P<0.001$ (Student's t-test)

Fig. 2. Dose response curve for the effect of probenecid on cAMP levels in cisternal CSF obtained 120 min after IP application of the drug. $n=4$–6 rats, at 50 mg/kg body weight $n=2$. The accumulation is significant $P<0.01$ at 100 mg/kg, $P<0.001$ at 150 mg/kg



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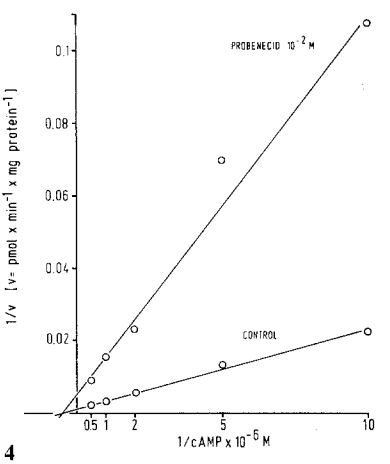

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Fig. 3. Effect of probenecid on the activity of a low K_m phosphodiesterase from rat brain homogenate. Each point represents the mean of two determinations, substrate concentration was 10^{-6}M

Fig. 4. Lineweaver-Burk plot of the activity of a low K_m phosphodiesterase from rat brain homogenate, $K_m=2.0\times10^{-6}$M, $V_{max}=10^{-3}$ pmol/min/mg protein. Non-competitive inhibition by probenecid

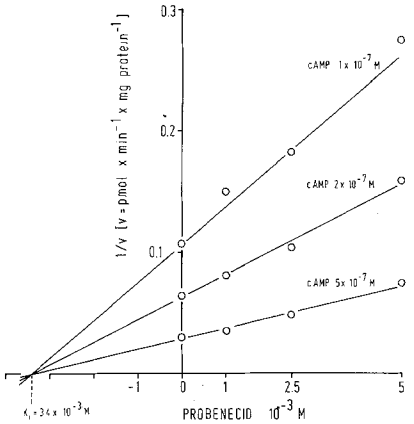

Fig. 5. Dixon plot of the inhibitory effect of probenecid on the activity of a low K_m phosphodiesterase from rat brain homogenate, inhibitor constant $K_i = 3.4 \times 10^{-3}$M

The probenecid dose required for maximum cAMP accumulation was 150 mg/kg body weight (Fig. 2). Up to a dose of 250 mg/kg no difference was found in the behaviour of the experimental compared to untreated animals. With 300 mg/kg, the animals became sleepy after 30 min and after 45 min they were lying motionless with increased muscle tone.

Probenecid at a dose of 200 mg/kg had no effect on the concentration of cAMP in serum or urine, and did not significantly alter the circadian rhythm of the cyclic nucleotide levels observed in these fluids (results not shown).

With a probenecid concentration of 0.5×10^{-3}M, an inhibitory effect of the drug was detectable on a low K_m fraction of the cyclic nucleotide *phosphodiesterase* from rat brain homogenate (Fig. 3). Lineweaver-Burk (Fig. 4) and Dixon plots (Fig. 5) demonstrate an enzyme inhibition of the non-competitve type with an inhibitor constant of 3.4×10^{-3}M.

Discussion

Dopamine and noradrenaline receptors of cerebral adenylate cyclase have been demonstrated in vivo and in vitro (Kebabian et al. 1971; Westermann 1973; Garelis and Neff 1974). In the rat inhibition of catecholamine synthesis by α-methyl-p-tyrosine (Cramer and Lindl 1972) and stimulation of catecholamine synthesis by L-DOPA (Kiessling et al. 1975) caused corresponding changes in cAMP concentrations in CSF. These findings suggest that CSF levels of cAMP reflect the activity of cerebral adenylate cyclase.

However in contrast to these experimental conditions rather small changes of cAMP concentrations have been found in CSF of patients with various neurological diseases (Cramer et al. 1973). This might be due to a carrier-mediated eliminating system from the CSF compartment, which masks the changes of cerebral cAMP synthesis under pathological or therapeutical conditions.

The rapid increase in the concentration of cAMP in CSF after probenecid administration suggests that the principle of the "probenecid test" is also valid for cAMP. In our experiments in rats a single IP injection of 150 mg/kg probenecid

caused a five-fold increase in cisternal cAMP. A quite similar accumulation of 5-HIAA was observed in the CSF of dogs after oral treatment with the same dose of probenecid (Guldberg et al. 1966). Sebens and Korf (1974) reported that IV administered cAMP does not cross the blood-brain barrier. Our results of virtually unchanged serum levels of cAMP after probenecid treatment support previons findings that elevated cAMP levels in the CSF do not originate from elevated blood levels.

In addition to the probenecid effect on the uptake of cyclic nucleotides by the choroid plexus (Hammers et al. 1977), we found an inhibition of the high affinity fraction of phosphodiesterase activity in rat brain homogenates characterized as a non-competitive effect with an inhibitor constant of 3.4×10^{-3}M.

Many substances have been found to inhibit phosphodiesterase activity, amongst them chemically unrelated agents such as methylxanthines, papaverine, diuretics, antibiotics and psychotropic drugs such as phenothiazines and benzodiazepines (reviewed by Appleman et al. 1973). Moreover, a series of new drugs have been developed with specifically potent inhibitory effects on phosphodiesterase (Dalton et al. 1970; Beavo et al. 1971). Compared with these drugs showing inhibitor constants of 10^{-6} to 10^{-4}M, the effect of probenecid is of low specifity. At high doses however, it has to be taken into account as a possible mode of action.

In conclusion probenecid causes a several fold increase in cAMP concentration in CSF in the rat similar to the earlier described effects on HVA and 5-HIAA in other experimental animals. The accumulation appears to be primarily due to transport inhibition from the CSF compartments into blood. These findings also confirm the validity of the "probenecid test" for cAMP in clinical neurology.

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