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PRELIMINARY COMMUNICATION

ANOMALOUS EFFECT OF PROBENECID ON RAT BRAIN PHENOLSULPHOTRANSFERASE

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It is generally accepted that 3-methoxy-4-hydroxyphenylethyleneglycol (MHPG)-sulphate is the main metabolite of noradrenaline in the brain of the rat and a number of other mammals [1]. The level of MHPG-sulphate in the brain is often regarded to reflect the noradrenergic activity. Experiments in which foreign compounds are injected intraperitoneally in the rat and in which after a certain period of time the MHPG-sulphate levels in the brain are measured, are considered to be of value in estimating the influence of the drug under study on the noradrenergic system in brain. Although a number of such studies has been carried out in which the effect of various drugs on the MHPG-sulphate content in brain tissue has been examined, the possibility that the drugs might influence the sulphating reaction itself has not yet been studied.

Probenecid (p-(dipropylsulfamoyl)benzoic acid) inhibits the active transport of acid compounds out of the brain. The drug has often been used to estimate the turnover of monoamines by determination of the rate of accumulation of acid metabolites in brain tissue and cerebrospinal fluid after treatment [2].

MHPG-sulphate is supposed to be eliminated from rat brain by a probenecid sensitive acid transport system, and the effects of probenecid treatment on MHPG-sulphate levels have been ascribed to inhibition of this transport system [3]. The possibility of a direct action of probenecid on the enzyme converting MHPG to MHPG-sulphate was, however, never investigated.

This sulphate conjugation is catalyzed by the enzyme phenolsulphotransferase (3'-phosphoadenylylsulphate: phenolsulphotransferase; EC 2.8.2.1). We here report the influence of probenecid on this reaction. Two methods are used for the enzyme assay, based on the use of two different substrates, 4-methylumbelliferone and MHPG itself [4,5].

In these assays maximal enzyme activity is reached at a substrate concentration of 0.2 mM; at 0.025 mM the enzyme is not saturated and at 0.5 mM substrate inhibition occurs. By addition of different concentrations of probenecid (a gift of Merck, Sharp and Dohme) to the incubation mixtures nor the internal standards nor the blanks used in the experiments were influenced. The enzyme preparation from rat brain was a 10% w/v homogenate in 0.32 M sucrose. As sulphate donor a crude preparation of 3'-phosphoadenosine-5'-phosphosulphate (PAPS, "active sulphate") was used, generated by incubation of a lyophilized rat liver extract with ATP and sulphate in the presence of magnesium ions.

Table 1. Effect of probenecid on rat brain phenolsulphotransferase.

probenecid mM	substrate concentration mM									
	4-methylumbelliferone					MHPG				
	0.025	0.05	0.10	0.20	0.50	0.025	0.05	0.10	0.20	0.50
0	85	109	106	100	80	63	80	92	100	78
1	72	98	117	130±5	124	54	83	131	130±4	131
2	58	86	100	130±7	153	39	71	102	126±8	148
5	27	38	55	68±5	120	18	34	57	84±4	150
No. of exp.	2	2	3	10	2	2	2	3	4	2

Effects of variation in concentration of probenecid and substrates are presented as percentages of the activities obtained at 0.2 mM substrate without added probenecid. The amount of product formed at this concentration were 300 nmoles/g/h and 340 nmoles/g/h for 4-methylumbelliferone and MHPG respectively. When 4 or more experiments at the same concentration were performed the percentage is given with S.E.M.

The 3.0 ml incubation mixture consisted of 2.0 ml PAPS-preparation, 0.5 ml rat brain homogenate and a final concentration of Triton X-100 of 0.16%, tris-HCl buffer pH 7.4 0.4 M, KH_2PO_4 19 mM, and substrates and probenecid as given in Table 1. Incubations were performed for 30 min at 37^{o} C.

After addition of different concentrations of probenecid to the incubation mixture, at the optimal substrate concentration of 0.2 mM, a significant increase in the amount of formed product was observed, at probenecid concentrations of 1.0 and 2.0 mM. A decrease, however, was found at the higher probenecid concentration. Probenecid itself does not interfere with the isolation nor with the determination of the formed product in both assay methods for phenolsulphotransferase.

We also investigated the effect of probenecid on the phenolsulphotransferase activities at higher and lower substrate concentrations. At the lower concentrations of both substrates the inhibitory effect of probenecid predominates, at the higher substrate concentration an activating action is observed up to 5 mM probenecid. The results are summarized in Table 1.

We tried to find out whether this effect could be attributed to other factors than a direct action on the enzyme itself. The effect is also observed after the use of a relatively pure, chemically synthesized [6], preparation of PAPS. Deletion of Triton X-100, addition of EDTA, the use of recrystallized probenecid did not alter the effect. The same action of probenecid is also found for enzyme preparations consisting of high speed supernatant or high speed pellets from rat brain tissue in 0.32 M sucrose, as is the fact after application of hypo-osmotic shock. So we cannot ascribe the effect to an action of probenecid on intact membrane structures.

We tried to find out whether we could observe the same results when using a rat liver preparation instead of rat brain. In suitably diluted rat liver homogenates we observe only a concentration dependent inhibitory action of probenecid on phenolsulphotransferase.

Probenecid appears to possess an inhibitory and an activating action on

rat brain phenolsulphotransferase, depending on its own concentration and on that of the substrate, an effect that is not observed for the rat liver enzyme. To what extent these effects influence the MHPG-sulphate concentration in brain after probenecid in vivo cannot be anticipated. But, in view of the high doses of probenecid administered to patients and animals, these findings may have practical implications with regard to the measurement of MHPG-sulphate after probenecid treatment and they may complicate the interpretation of the results of in vivo experiments using this drug.

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