Biochimica et Biophysica Acta, 419 (1976) 309–313

BBA 77174

THE INHIBITOR EFFECT OF PROBENECID AND STRUCTURAL ANALOGUES ON ORGANIC ANIONS AND CHLORIDE PERMEABILITIES IN OX ERYTHROCYTES

R MOTAIS and J L COUSIN

Laboratoire de Physiologie Cellulaire, Faculte des Sciences de Nice, Parc Valrose, 06034 Nice Cedex (France)

(Received July 7th, 1975)

SUMMARY

Probenecid inhibits anion movements (organic anions and chloride) in ox erythrocytes The I_{50} is 4 10^{-5} M Structural analogues such as carinamide, *p*-carboxybenzene sulfonamide and *p*-carboxy *N,N* diethyl benzene sulfonamide, which are drugs of the sulfonamide class, were also found to inhibit anion transport These results reinforce the previously discussed view based on structural considerations, that sulfonamides act on the red cell membrane as competitors of anion transport It is possible that probenecid and carinamide act in a similar way in the kidney

---

Renal tubular excretion of organic anions such as hippurate is an active transport process which is inhibited by probenecid ("Benemid") The mechanism of inhibition, however, is still not clear [1]

In the erythrocyte, organic anions also permeate by means of a specific transport mechanism and not by free diffusion through an aqueous channel limited by positive charges [2] The purpose of the present study was firstly to test whether probenecid and analogues similarly impair the penetration of organic anions into erythrocytes and secondly to consider the mechanism by which inhibition occurs

The experiments were performed on ox erythrocytes As prototypical of substrates of the organic anion transport system we used oxalate for the unidirectional tracer experiments and pyruvate for the net flux experiments, in which a high concentration of substrate (160 mM) is needed Both of these anions have been previously demonstrated [2] to permeate the ox erythrocyte membrane through the organic anion transport mechanism The rate of organic anion transfer was measured under three different experimental conditions

(*a*) *Net exchange of extracellular pyruvate with intracellular bicarbonate* (pyr_{ext}^-/HCO_{3int}^-) when erythrocytes are suspended in an isotonic solution of ammonium puruvate a pyr_{ext}^-/HCO_{3int}^- exchange occurs which induces haemolysis, [3] The speed of haemolysis, measured by absorbance changes of the solution represents the penetration rate of the pyruvate anion [2]

(*b*) *Net exchange of extracellular pyruvate with intracellular chloride* (pyr_{ext}^- / Cl_{int}^-) when erythrocytes are suspended in an isotonic sodium pyruvate solution, a pyr_{ext}^-/Cl_{int}^- exchange occurs The net efflux of chloride, which is followed in the external medium by titration (Cotlove chloridometer), measures the net influx of pyruvate

(*c*) *Tracer efflux of oxalate at Donnan equilibrium which measures the rate of oxalate self exchange* ($oxal_{ext}^-/oxal_{int}^-$) The cells were loaded with [^{14}C]oxalate by incubation for 2 h at 20 °C in radioactive suspension medium (10 mM sodium oxalate, 140 mM NaCl, 10 mM KCl, 20 mM Tris-HCl) Then, after centrifugation, the packed cells were divided into different batches which are resuspended (hematocrit 50 %) in the incubation medium at exactly the same specific activity with or without probenecid or analogue (10^{-3} M) and incubated overnight at 0 C to allow the drug to equilibrate fully with the cells Subsequently the cells were centrifuged, the sediment cooled to 0 °C and mixed at that temperature (± 0 01 C) with unlabelled medium of the same composition as that in which they were previously suspended (hematocrit 0 5 %) This induced the release of radioactivity into the medium while the system was at Donnan equilibrium with respect to non radioactive anion species (the pH at all temperatures was adjusted to 7 4) The appearance of radioactivity in the medium was measured by the technique of Dalmark and Wieth [4] but using an automatic collector of samples [5]

Figs 1 to 3 show that probenecid at 10^{-3} M inhibits organic anion movements both in conditions of Donnan equilibrium and under non-equilibrium conditions At equilibrium the inhibitory activity of the drug expressed as the I_{50}, i e the molar concentration which inhibits the anion transport by 50 %, was found to be 4 10^{-5} M In the same conditions no effect can be observed on cation transport It can be seen in Table I that structural analogues of probenecid, such as carinamide and *p*-carboxybenzene sulfonamide, also strongly decrease organic anion exchange It is noteworthy that all these drugs are of the sulfonamide class and that carinamide was the first drug used in renal physiology to inhibit organic anion transport

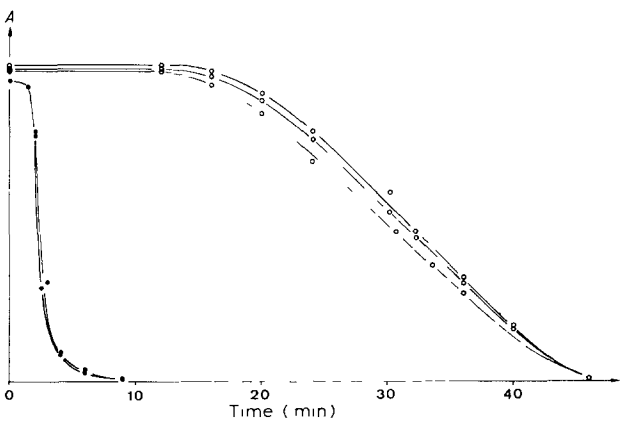


Fig 1 Influence of probenecid (10^{-3} M) on pyr_{ext}^-/HCO_{3int}^- exchange the time course of hemolysis, followed by the decrease in absorbance, of a suspension of ox erythrocytes in an isotonic ammonium pyruvate solution The erythrocytes were not preincubated with probenecid, temperature 34 °C ●, control (2 experiments), ○, probenecid (3 experiments)

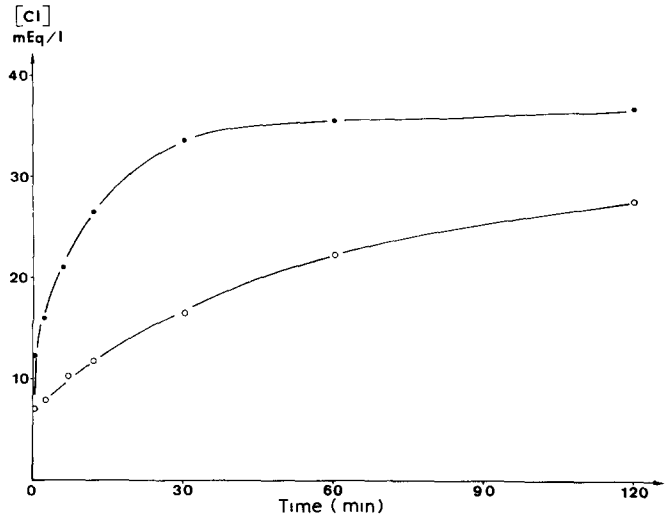


Fig 2 Influence of probenecid (10^{-3} M) on exchange of extracellular pyruvate with cellular chloride Hematocrit 37 per cent, 10 °C Erythrocytes were preincubated with probenecid ●, control, ○, probenecid

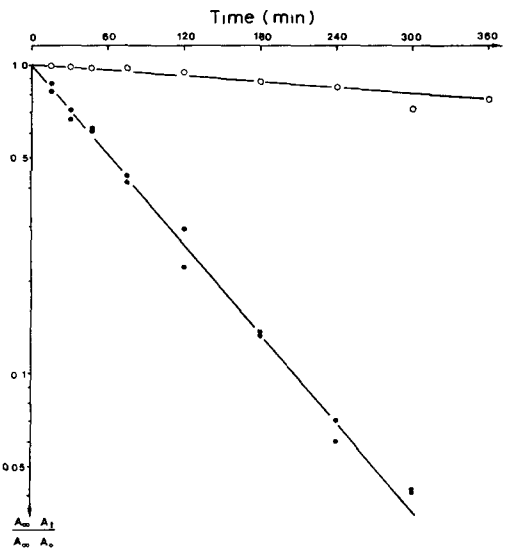
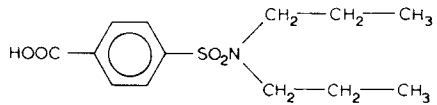
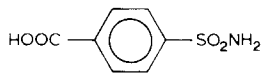
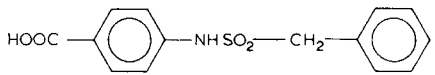
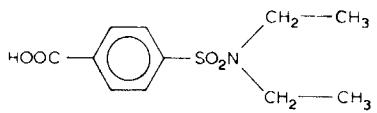


Fig 3 Inhibitory effect of probenecid (10^{-3} M) on oxalate transport measured under steady state conditions ●, control cells, ○, probenecid treated cells The rate coefficients of oxalate exchange were calculated from the slope of the graph of ln $(A_\infty{}^- A_t/A_\infty{}^- A_0)$ vs time (in minutes) A_∞, A_t and A_0 are the concentrations of isotope in the external medium at equilibrium, time t and 0 respectively

TABLE I

Effect of Probenecid and analogues (10^{-3} M) on oxalate and chloride transports I_{50} was only measured for chloride transport Cl^- self exchanges were measured as described in ref 5

Formula	Compounds	Oxalate exchange		chloride exchange		
		Rate coefficient (min^{-1})	% inhibition	Rate coefficient (min^{-1})	% inhibition	I_{50} (mol/l)
	Control	114 10^{-4}		3 36		
	Probenecid	7 10^{-4}	94	0 24	93	4 10^{-5}
	*p*-carboxy benzenesulfonamide	15 10^{-4}	87	0 40	88	10^{-4}
	carinamide	25 10^{-4}	78	0 97	71	3 5 10^{-4}
	*p*-carboxy-*N*,*N*-diethyl benzenesulfonamide			0 37	89	6 10^{-5}

It is of interest to consider the mode of action of these drugs on the erythrocyte anion transport system within the framework of the available data on this system It has been demonstrated that some carbonic anhydrase inhibitors of the sulfonamide class inhibit anion movements at the membrane level [6–7] The sulfonamide group (SO_2NH_2) of these drugs could satisfy the structural requirements for organic anion transfer through the erythrocyte membrane Indeed, interaction between anion and membrane receptor, allowing for translocation, requires at least a 3 point attachment involving 3 oxygen atoms on the anion which react with complementary loci on the receptor to form ionic and hydrogen bonds [2] Such a three point attachment can potentially be made by sulfonamide group because the electronegativities of oxygen and of nitrogen are similar On this basis a sulfonamide could act as a competitor on the receptor [6] The present results, which show that the tested sulfonamides inhibit the self exchange of oxalate, and data obtained with a large series of unsubstituted and substituted sulfonamides, strongly support this hypothesis

Since there are certain analogies between the renal effects of diuretics (sulfonamides [5-7], ethacrynic acid [8]) and their anionic inhibitory potency in the erythrocyte, it is possible that the mechanism whereby probenecid and carinamide inhibit organic anions in kidney is similar to that in the erythrocyte

Previous results showed that in ox erythrocytes the transport of chloride and organic anions is influenced in a strictly identical manner by a whole series of chemical substances [5–8] and by temperature variation [5], making it reasonable to postulate that in the ox erythrocyte the rate of transfer of chloride and organic anions is controlled by the same membrane component The data in Table I reinforce this assumption since the relative inhibitory potencies of the various drugs are identical for chloride and oxalate transports

ACKNOWLEDGEMENT

Supported by D G R S T (Contrat no 73-7-1521), C N R S (Physiologie des membranes E R A No 495) and the Département de Biologie du C E A

REFERENCES

1 Goldberg, M (1973) Handbook of Physiology Renal Physiology, pp 1003–1031, American Physiology Society, Washington
2 Aubert, L and Motais, R (1975) J Physiol 246, 159–179
3 Jacobs, M H and Stewart, D R (1942) J Gen Physiol 25, 539–552
4 Dalmark, M and Wieth, J O (1972) J Physiol 224, 583–610
5 Cousin, J L and Motais, R , J Physiol in press
6 Cousin, J L , Motais, R and Sola, F , J Physiol in press
7 Motais, R , Cousin, J L and Sola, F (1975) C R Acad Sc (Paris) 280, 1119–1122
8 Motais, R and Cousin, J L , Am J Physiol , submitted for publication