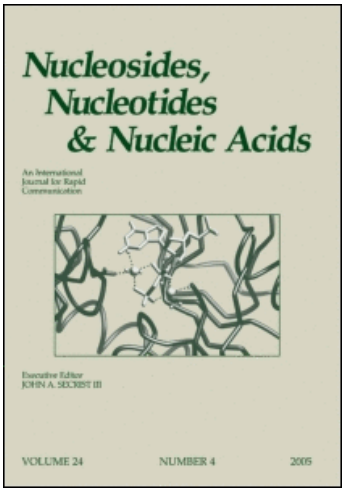

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THE INHIBITION OF NUCLEOSIDE TRANSPORT IN HUMAN ERYTHROCYTES BY MIOFLAZINE ANALOGUES

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Kinetic analysis of the transport protein (both influx and efflux), usually performed with radiolabelled nucleosides such as adenosine and uridine, has provided a wealth of information regarding the various kinetic and equilibrium parameters (1).
Here we report on the inhibition of [^{3}H]uridine transport in human erythrocytes by a new series of compounds related to lidoflazine and mioflazine. To determine the K_m and V_{max} values of the influx process as well the IC_{50} values of the compounds an oil layer centrifugation method was used (2). In this method the uptake of uridine by erythrocytes is stopped by means of fast centrifugation so that the heavier erythrocytes are forced through the oil to the bottom in contrast to the aqueous medium containing [^{3}H]uridine. Under our conditions we were able to measure uptake rates up from 3 seconds. All measurements were performed at 25°C.

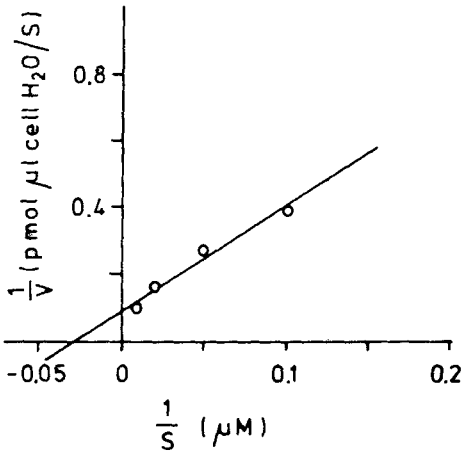

Fig.1: Lineweaver-Burk plot

Fig.1 shows the Lineweaver-Burk transformation of the kinetic data yielding a K_m value of 36 μM and a V_{max} of 11 pmol/μl cell H_2O/s. In the inhibition of the [^{3}H]uridine transport on erythrocytes all the compounds proved to be effective but a remarkable phenomenon showed up. On simultaneous addition of the compounds with 20 μM [^{3}H]uridine (uptake for 20 s) higher concentrations were needed to block the carrier than when the compounds were preincubated for 1 hour with the erythrocytes. Fig.2 shows the effect of two compounds with and without preincubation.

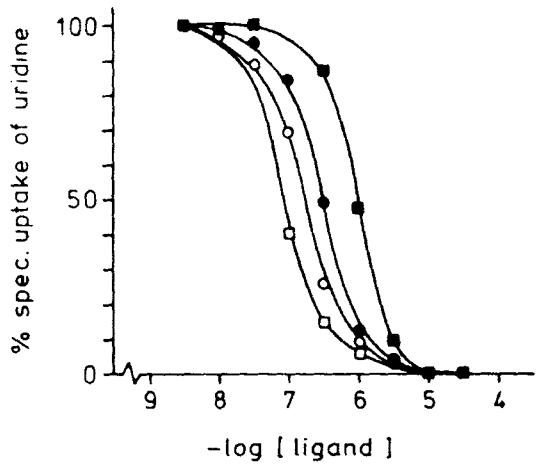

Fig.2: Inhibition curve of uridine uptake; ○,● =soluflazine, □,■=mioflazine. The filled symbols are without and the open symbols with preincubation.

Without preincubation soluflazine is more active than mioflazine whereas the reverse is true on preincubation, mainly due to the huge increase in activity of mioflazine. The results for the other compounds and of the reference compounds dilazep, dipyridamole, nitrobenzylthioinosine and soluflazine are summarized in Table 1, together with data from phosphate release experiments, as obtained in an earlier study (3).

Table 1: The structure of the tested compounds with their IC_{50} values (nM) for inhibition of uridine uptake without and with preincubation and their EC_{50} values (nM) for phosphate release.

	IC_{50} (nM)		EC_{50} (nM)
	- preincubation	+ preincubation	
Lidoflazine	1930 ± 130	246 ± 77	400
Mioflazine	665 ± 116	119 ± 36	83
R 51975	589 ± 128	97.5 ± 13.1	230
R 53531	893 ± 302	34.1 ± 13.4	26
R 75231	268 ± 71	12.9 ± 3.1	17
R 73796	414 ± 13	25.2 ± 1.3	13
R 73335	242 ± 94	34.3 ± 14.9	14
Dipyridamole	168 ± 37	278 ± 85	360
Soluflazine	315 ± 33	173 ± 29	130
Nitrobenzylthioinosine	34.9 ± 8.9	1.89 ± 0.42	95
Dilazep	268 ± 36	10.7 ± 1.5	17

Thus, R75231 proved to be the most active compound within the mioflazine series. Moreover, the activity of the reference compound NBI was also largely dependent on preincubation and appeared to be dissociated from its effects in the phosphate release experiments.

CONCLUSIONS

- R75231 was the most potent blocker within the new series of compounds.
- Only after preincubation all compounds except dipyridamole display full activity in preventing uridine transport at lower concentrations, comparable to data from the phosphate release experiments.
- Since in the series the most hydrophilic compound soluflazine does not show such large dependency on preincubation the lipophilic character of the inhibitors might be important.

(1) A.R.P. Paterson et al. In D.M. Paton (ed), Methods used in Adenosine Research, Plenum Press, NY, 1985, 165.
(2) R.M. Wohlhueter et al. Methods Cell.Biol. 20 (1978),211.
(3) A.P. Ijzerman et al. Eur. J. Pharmacol. Mol. Pharmacol. Sect., 172 (1989) 272.