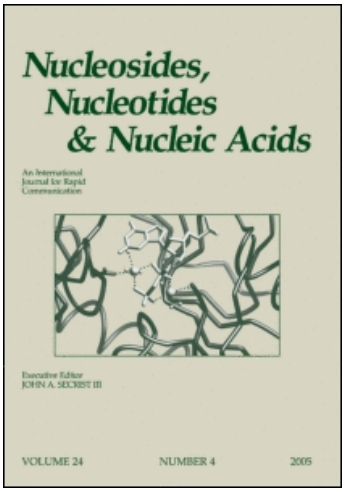

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Affinity of Ionic Species of Nucleoside Transport Protein Inhibitors

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AFFINITY OF IONIC SPECIES OF NUCLEOSIDE TRANSPORT PROTEIN INHIBITORS

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A class of very potent nucleoside transport inhibitors is present in two molecular forms around physiological pH. We investigated whether the monoprotonated or the unionized species of these molecules binds to this carrier protein with higher affinity.

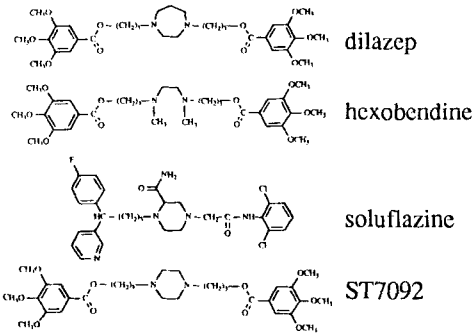

FIG.1. Chemical structures of the nucleoside transport protein inhibitors studied

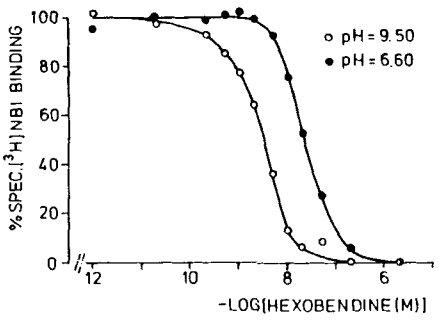

FIG.2. Displacement of [^{3}H]NBI by hexobendine at pH 6.60 (●) and at pH 9.50 (o)

Membrane preparations from calf lung tissue were obtained by differential centrifugation of the tissue homogenate. The specific equilibrium binding of [^{3}H]nitrobenzylthioinosine (NBI) to the membranes at three pH values was saturable and reversible, displaying lower affinity and higher capacity at pH 6.60 when compared to pH 7.40 and pH 9.50 (Table 1).

	pH 6.60	pH 7.40	pH 9.50
K_D (nmol/l)	0.66 ± 0.05	0.65 ± 0.05	0.45 ± 0.04
B_{max} (pmol/mg protein)	3.8 ± 0.2	2.5 ± 0.2	2.3 ± 0.1

TABLE 1. pH-dependency of [^{3}H]NBI saturation (n=3)

All inhibitors are capable of displacing [^{3}H]NBI binding at both pH values, although to a lesser extent at pH 6.60 (Fig.2 and Table 2).

Degradation of the compounds during the incubation with the calf lung membrane preparation was determined. The inclusion of 100 μmol/l physostigmine in the incubation mixture used in degradation experiments largely prevents this degradation. In Table 2 the degradation data for the four compounds at pH 6.60 as well as pH 9.50 in the presence of physostigmine are listed. Obtained K_i values were corrected for degradation and are listed in Table 2 also.

	degradation (% intact molecules)		K_i values (nmol/l)	
	pH 6.60	pH 9.50	pH 6.60	pH 9.50
dilazep	98.2	89.4	2.23	0.68
hexobendine	98.6	91.7	9.95	0.98
soluflazine	97.1	91.8	2.38	1.65
ST7092	97.5	85.9	2.93	0.42

TABLE 2. Degradation and K_i values corrected for degradation (n=3)

From the pK_a values of the compounds the fractions of both neutral and monoprotonated species at the two pH values were calculated. Combining these data with the apparent K_i values, we obtained the 'true' affinities of both species (Table 3).

	$K_{i,N}$ (nM)	$K_{i,P}$ (nM)	$\%N_{6.6}$	$\%P_{6.6}$	$\%N_{9.5}$	$\%P_{9.5}$
dilazep	0.58	2.35	2.1	94.6	94.7	5.3
hexobendine	0.12	10.2	1.3	97.9	91.5	8.5
soluflazine	1.64	7.79	87.5	12.1	99.98	0.02
ST7092	0.27	3.10	2.1	94.6	94.7	5.3

TABLE 3. Calculated affinities of neutral (N) and monoprotonated (P) species from the percentage species at pH 6.60 and pH 9.50

Thus, the neutral species of the inhibitors show higher affinity for the nucleoside transport protein.