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Nitrobenzylthioinosine inhibition of adenosine uptake in guinea-pig brain

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Nitrobenzylthioinosine (NBMPR) binds to rat and guinea-pig brain membrane fractions at a saturable, high affinity site (K_D values 0·05–0·25 nM) (Hammond & Clanachan 1982; Marangos et al 1982a,b; Wu & Phillis 1982) which has been thought to represent the adenosine transporter protein, since NBMPR and related compounds are potent inhibitors of nucleoside transport in various non-nervous animal cells (Paterson et al 1980). NBMPR has an IC50 for inhibition of nucleoside transport in human erythrocytes of $1·5 \times 10^{-8}$ M (Paul et al 1975). 2-Amino-6[(4-nitrobenzyl)thio] guanosine (NBTGR) is an even more potent inhibitor of erythrocyte nucleoside transport with an IC50 of $5·8 \times 10^{-9}$ M (Paul et al 1975).

Barberis et al (1981) in one experiment on guinea-pig cortical synaptosomes observed a 77% inhibition of adenosine uptake by NBMPR (16·3 nM) but NBTGR caused only 33% inhibition at 1 μM. We failed to confirm the potent inhibitory activity of these purine derivatives on adenosine uptake using rat brain cerebral cortical synaptosomes. NBMPR had an IC50 of $5·9 \times 10^{-5}$ M (K_i, $3·0 \times 10^{-5}$ M) and NTBGR an IC50 of $5·0 \times 10^{-5}$ M (Bender et al 1981). The discrepancy between the dissociation constant for NBMPR binding in rat brain ($0·05 \times 10^{-9}$ M, Wu & Phillis 1982) and its K_i value for inhibition of adenosine uptake ($3·0 \times 10^{-5}$ M) raises concern about the nature of the high affinity binding site for NBMPR on brain membranes. If this site is on the adenosine transporter protein, it may be located on some region which is associated with, but is not essential for, transport function.

Table 1. Inhibition by nitrobenzylthioinosine of adenosine uptake by guinea-pig brain cerebral cortical synaptosomes. Synaptosomes (0·5 mg protein) were preincubated in the presence or absence of nitrobenzylthioinosine at 37 or 20 °C for 2 min. Uptake was initiated by the addition of [^{3}H] adenosine to the medium at a final concentration of 1×10^{-6} M. Incubation was for 30 s. The data presented as means ±s.e. and the results of two experiments conducted in quadruplicate. The IC50 values referred to in the text were obtained by extrapolation from the dose response curves.

Concn (M)	% Control uptake 37 °C	20 °C
0	100	100
10^{-9}	81·2 ± 1·7	85·3 ± 3·2
5×10^{-9}	76·8 ± 1·8	73·4 ± 3·6
10^{-8}	69·8 ± 4·2	73·9 ± 2·2
5×10^{-8}	—	67·3 ± 2·7
10^{-7}	70·5 ± 2·6	61·9 ± 4·1
5×10^{-7}	66·5 ± 2·42	65·8 ± 1·1
10^{-6}	60·5 ± 5·3	52·5 ± 1·6

Because of the discrepancies between our data for NBMPR inhibition of adenosine uptake in rat brain (Bender et al 1981) and that for guinea-pig brain cortical synaptosomes (Barberis et al 1981), we have examined the guinea-pig brain preparation at two temperatures (in Table 1). The experiment at 20 °C was to make a valid comparison with binding studies at 20–22 °C (Patel et al 1982; Hammond & Clanachan 1982; Marangos et al 1982). It is clear that the concentration of NBMPR required for 50% inhibition of adenosine uptake is in excess of 10^{-6} M ($1·7 \times 10^{-6}$ and $4·5 \times 10^{-6}$ M at 20 and 37 °C respectively). The values are 10^2 times greater than those reported for the inhibition of human erythrocytes (Paul et al 1975), and 10^4 times greater than the K_D for NBMPR binding to guinea-pig brain-membranes (0·25 nM; Hammond & Clanachan 1982). We suggest that the use of NBMPR as a high affinity probe for the adenosine transport site should be viewed with some caution and that the failure of various adenosine uptake inhibitors to displace NBMPR binding to brain membranes (Wu & Phillis 1982; Patel et al 1982; Hammond & Clanachan 1982) may be a consequence of the specific localization of its binding site on brain membranes.

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