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INHIBITION OF α-AMINOISOBUTYRIC ACID UPTAKE BY DIISOTHIOCYANOSTILBENEDISULPHONIC ACID IN THE AMPHIBIAN CORNEA

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Summary

α-Aminoisobutyric acid accumulation of the toad's (*Bufo marinus*) cornea and lens is inhibited by 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid. This effect is seen at pH 8.4; at pH 7.4 a small increase in aminoisobutyric acid uptake was observed. Efflux of aminoisobutyric acid is unchanged by diisothiocyanostilbenedisulphonic acid at either pH. The inhibitory effect of diisothiocyanostilbenedisulphonic acid on aminoisobutyric acid accumulation appears to reflect a direct action on membrane mechanisms that mediate its influx.

The cornea of the toad, *Bufo marinus*, actively accumulates the non-metabolizable amino acid, α-aminoisobutyric acid, in vitro [1]. Uptake is greater at pH 8.4, which is similar to that of body fluids of amphibians [2], than at pH 7.4. We have found that aminoisobutyric acid uptake by the toad's cornea is inhibited by DIDS at the higher pH. DIDS (and its analogue SITS) inhibits Cl^-/HCO_3^- exchange across the erythrocyte cell membrane [3]. It does not enter cells but acts on the outer surface where it binds, irreversibly, to a component called the 'band 3' protein. DIDS has also been shown to inhibit Cl^- (but not Na^+) transport across the toad cornea [4] and SITS has a similar effect

Abbreviations: DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulphonic acid; SITS, 4-acetamido-4′-isothiocyanostilbene-2,2′-stilbenedisulphonic acid.

TABLE I

EFFECTS OF DIDS ON ACCUMULATION OF AMINOISOBUTYRIC ACID BY THE TOAD CORNEA AND LENS IN VITRO

The tissues were incubated in 5 ml of Conway solution containing (mM): Na^+, 104; K^+, 2.5; Ca^{2+}, 1.0; Mg^{2+}, 1.2; Cl^-, 74.5; HCO_3^-, 25; SO_4^{2-}, 1.8; HPO_4^{2-}, 2.9; gluconate, 1.0; glucose, 26.0. It was aerated to give a pH of 8.4 or bubbled with 5% CO_2 to give pH 7.4. Corneas were incubated for 3 h in these solutions containing 0.1 mM aminoisobutyric acid (AIB) and 0.2 μCi of amino[^{14}C]isobutyric acid (New England Nuclear). At the end of the experiment the aminoisobutyric acid was eluted in 0.5 M HCl and the radioactivity counted in a Beckman LSC-100 apparatus. The results are given as mean ±S.E. for six pairs of corneas or lenses, one being the control, the other exposed to DIDS.

	Tissue-to-medium concentration ratio		
	Control	+DIDS ($5 \cdot 10^{-3}$ M)	*P* for difference
Cornea			
pH 8.4 (AIB)	2.42 ± 0.20	1.63 ± 0.08	<0.01
pH 8.4 (alanine)	4.51 ± 0.09	2.48 ± 0.17	<0.02
pH 7.4 (AIB)	1.53 ± 0.09	2.59 ± 0.12	<0.01
Lens			
pH 8.4 (AIB)	2.42 ± 0.20	0.82 ± 0.21	<0.01
pH 8.4 (alanine)	7.04 ± 0.95	4.22 ± 0.33	<0.01
pH 7.4 (AIB)	1.07 ± 0.05	2.06 ± 0.18	<0.001

on the turtle urinary bladder [5]. It has recently been shown that SITS can also inhibit organic anion transport, as it blocks secretion of *p*-aminohippurate secretion in the renal tubule [6,7].

Aminoisobutyric acid accumulation in the toad's cornea, and crystalline lens, was measured in vitro (Table I). At a pH of 8.4 this was inhibited by DIDS. Alanine uptake was also reduced. At pH 7.4, however, this effect was not seen, and surprisingly, aminoisobutyric acid accumulation was then somewhat increased in both tissues. Efflux of aminoisobutyric acid from the cornea and lens was unaffected by DIDS at either pH (Table II).

TABLE II

EFFLUX OF AMINOISOBUTYRIC ACID FROM THE TOAD CORNEA AND LENS IN THE PRESENCE AND ABSENCE OF DIDS

Corneas and lenses were preloaded for 3 and 0.75 h, respectively, in Conway solution containing 0.1 mM aminoisobutyric acid and 0.2 μCi/ml amino[^{14}C]isobutyric acid. They were then transferred at intervals through a series of Conway solution containing no aminoisobutyric acid so that the rate of exodus of aminoisobutyric acid from the preloaded tissues could be determined. The results are expressed as the total loss of aminoisobutyric acid after 4 h. The values are means ±S.E. for six paired tissues, one a control, the other exposed to DIDS.

	Efflux (nmol/mg per 4 h)	
	Control	+DIDS ($5 \cdot 10^{-3}$ M)
Cornea		
pH 8.4	106 ± 5	92 ± 6
pH 7.4	120 ± 4	110 ± 4
Lens		
pH 8.4	24.1 ± 3.8	24.4 ± 2.6
pH 7.4	17.6 ± 2.3	19.1 ± 1.7

TABLE III

EFFECT OF DIDS ON AMINOISOBUTYRIC ACID ACCUMULATION IN CORNEAS MOUNTED IN DIVIDED, USSING-TYPE, CHAMBERS

Incubation time was 3 h. The tissues were electrically short-circuited with an automatic voltage clamp. Results are given as means ±S.E. for six paired corneas, one used as the control, the other exposed to DIDS. n.s., not statistically significant, $P > 0.05$.

	Tissue-to-medium concentration ratio		
	Control	+DIDS (10^{-5} M)	P for difference
pH 8.4			
DIDS on tear side	6.24 ± 0.28	5.86 ± 0.40	n.s.
DIDS on aqueous side	4.29 ± 0.31	2.77 ± 0.42	<0.01
pH 7.4			
DIDS on tear side	1.24 ± 0.05	1.44 ± 0.05	<0.01
DIDS on aqueous side	1.14 ± 0.07	2.06 ± 0.06	<0.01

DIDS inhibits Cl^- transport across the cornea when it is present on either its tear or aqueous side, suggesting the presence of an anion exchange system on both surfaces [4]. Uptake of aminoisobutyric acid only occurs across the aqueous side [8]. It is possible that the inhibitory effect of DIDS on aminoisobutyric acid uptake is an indirect result of the change in Cl^- transport. However, stilbene was only found to block aminoisobutyric acid accumulation when present on the aqueous side (Table III). In addition, bumetanide (10^{-5} M) which also blocks Cl^- transport across the toad cornea [9] had no effect on aminoisobutyric acid uptake. In 3 h the tissue-to-medium concentration ratio was 2.41 ± 0.30 in the control and 2.28 ± 0.20 in the paired corneas (six pairs) exposed to bumetanide.

The action of DIDS in increasing aminoisobutyric acid accumulation at pH 7.4 appears to be indirect, as it increases aminoisobutyric acid accumulation when present on either side of the cornea (tear or aqueous (Table III)). An indirect action may reflect a change in intracellular ion levels, such as H^+ and HCO_3^-, that create conditions more favourable to aminoisobutyric acid uptake.

DIDS may be directly interfering with the process of aminoisobutyric acid influx into the cornea and lens by interacting with a component in their cell membranes. However, it it interesting that this effect is only observed at a pH of 8.4. It is possible that DIDS, due to greater dissociation, is more effective at this pH. Alternatively, about 4% of the aminoisobutyric acid is in an anionic form at pH 8.4 and this may influence the process of its transport. Accumulation of aminoisobutyric acid under such conditions thus could partly involve an organic anion-like transport mechanism, which would be consistent with the inhibitory effect of DIDS.

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