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# GLUCOSE TRANSPORT IN FISH ERYTHROCYTES: VARIABLE CYTOCHALASIN-B-SENSITIVE HEXOSE TRANSPORT ACTIVITY IN THE COMMON EEL (*ANGUILLA JAPONICA*) AND TRANSPORT DEFICIENCY IN THE PADDYFIELD EEL (*MONOPTERUS ALBUS*) AND RAINBOW TROUT (*SALMO GAIRDNERI*)

BY CHUNG-MING TSE* AND JAMES D. YOUNG†

*Department of Biochemistry, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong*

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## Summary

Erythrocytes from individual common eels (*Anguilla japonica* Temminck and Schlegel) exhibited widely variable initial rates of cytochalasin-B-sensitive 3-*O*-methyl-D-glucose (3-OMG) zero-*trans* influx, in the range 0–19.5 mmol l-cells$^{-1}$ h$^{-1}$ (5 mmol l$^{-1}$ extracellular concentration at 20°C, 50 animals tested). Storage of cells at 4°C in a glucose-containing medium for up to 72 h had no effect on 3-OMG uptake, and there was no correlation between the sugar permeabilities of erythrocytes from different fish and intracellular ATP levels. Adrenaline and noradrenaline increased cytochalasin-B-sensitive 3-OMG transport activity; half-maximal stimulation occurred at catecholamine concentrations in the region of 1 μmol l$^{-1}$. This catecholamine-induced stimulation of sugar transport appeared to be independent of the basal cytochalasin-B-sensitive 3-OMG permeability of the cells. Kinetically, catecholamines increased the $V_m$ of transport without changing the apparent $K_m$ (approx. 1.4 mmol l$^{-1}$). Saturable 3-OMG influx was inhibited by phloretin, D-glucose, D-deoxyglucose and D-galactose, but not by D-fructose and L-glucose. Transporter stereoselectivity was confirmed by direct measurements of D- and L-glucose uptake. Erythrocytes from two other fish species, *Monopterus albus* Richardson (paddyfield eel) and *Salmo gairdneri* Richardson (rainbow trout), unlike those from the common eel, were uniformly deficient with respect to cytochalasin-B-sensitive 3-OMG and D-glucose transport activity. Catecholamines had no effect on sugar uptake in these species.

## Introduction

Equilibrative transport of D-glucose has been studied extensively in both

* Present address: Department of Medicine, GI Division, The Johns Hopkins University School of Medicine, 725, Wolfe Street, Hungterian 503, Baltimore, MD 21205, USA.
† To whom correspondence should be addressed.

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mammalian and avian erythrocytes. Human erythrocytes and foetal erythrocytes from other mammalian species are capable of rapid, stereoselective transport of glucose (Widdas, 1980; Kim, 1983). In contrast, erythrocytes from the pig are deficient in glucose transport activity (Kim and McManus, 1971; Young *et al.* 1985), the cells relying on plasma inosine as their physiological energy substrate (Young *et al.* 1985, 1986). Mature, adult-type erythrocytes from sheep and other non-primate mammalian species exhibit intermediate rates of stereospecific glucose transport (Mooney and Young, 1978; Kim, 1983; Wagner *et al.* 1984). In most cases, glucose uptake is inhibited by cytochalasin B. However, in rabbit erythrocytes, carrier-mediated D-glucose transport is insensitive to cytochalasin B (Albert, 1984). At the molecular level, the human erythrocyte glucose transporter has been purified and its amino acid sequence determined (Mueckler *et al.* 1985). A stereoselective, cytochalasin-B-sensitive glucose transporter has also been characterized in avian erythrocytes (Kregenow, 1975). Transport of glucose in these cells, in contrast to that in mammalian erythrocytes, is stimulated by anoxia and by catecholamines (Bihler *et al.* 1982; Simons, 1983*a*).

There is little published information about glucose transport in fish erythrocytes. Ingermann *et al.* (1984) reported a high level of cytochalasin-B-sensitive glucose transport activity in erythrocytes from the Pacific hagfish (*Eptatretus stouti*), a representative of the most primitive group of living vertebrates. In contrast, in an earlier study, Bolis *et al.* (1971) were unable to detect stereospecific glucose uptake in erythrocytes from the brown trout (*Salmo trutta*). We report here the characteristics of 3-*O*-methyl-D-glucose (3-OMG) and D- and L-glucose transport in erythrocytes from two fish species native to eastern Asia, the common eel (*Anguilla japonica*) and the paddyfield eel (*Monopterus albus*). For comparison, we have also studied 3-OMG transport in erythrocytes from the rainbow trout (*Salmo gairdneri*).

## Materials and methods

### *Fish*

Common eels (*Anguilla japonica*) and paddyfield eels (*Monopterus albus*) were obtained locally in Hong Kong and maintained in freshwater aquaria. *M. albus* belongs to a different order (Synbranchiformes) from *A. japonica*, but derives its name from its eel-like appearance and behaviour. Rainbow trout (*Salmo gairdneri*) were obtained from a commercial source in Cambridge, UK, and also maintained in freshwater aquaria. Fish were not fed while in captivity.

### *Cell collection and preparation*

Blood samples were obtained from the dorsal aorta of decapitated fish and collected into heparin. Erythrocytes were washed free of plasma by three washes in a medium containing 150 mmol $l^{-1}$ NaCl, 5 mmol $l^{-1}$ D-glucose and 15 mmol $l^{-1}$ Mops (pH 7.5 at 20°C). The buffy coat was discarded. The washed cells were resuspended to a haematocrit of approximately 10 % and left overnight at 4°C.

This was to ensure that the cells were at a steady state with respect to the incubation medium and not in a catecholamine-stimulated condition (Bourne and Cossins, 1982).

### *Haemoglobin estimation*

The haemoglobin contents of cell suspensions were determined by absorbance measurements of cyanmethaemoglobin [1:50 dilution with Drabkin's solution (100 mg $l^{-1}$ NaCN and 300 mg $l^{-1}$ $K_3Fe(CN)_6$ in distilled water)] at 540 nm. These values were used to estimate the volumes of cells in the original suspensions, calculated from an experimentally determined extinction coefficient of 218 for packed fish erythrocytes. There was no difference in the value of this coefficient among the three fish species studied.

### *ATP assays*

Erythrocyte ATP concentrations were determined by a firefly luciferin–luciferase assay (Brown, 1982).

### *Transport measurements*

The experiments were performed at 20°C using an isotonic incubation medium containing 150 mmol $l^{-1}$ NaCl and 15 mmol $l^{-1}$ Mops (pH 7.5). Uptake of 3-*O*-methyl-D-[U-$^{14}$C]glucose (Amersham International, Amersham, Bucks., UK), a non-metabolized D-glucose analogue, and D-[U-$^{14}$C]glucose and L-[1-$^{14}$C]glucose were initiated by mixing equal volumes (0.15 ml) of prewarmed cell suspension (haematocrit 20 %) with incubation medium containing the appropriate concentration of radioactive permeant (0.7 $\mu$Ci $ml^{-1}$). For incubations in the presence of cytochalasin B or phloretin, cells were equilibrated with inhibitor for 30 min at 20°C before addition of 3-*O*-methyl-D-[U-$^{14}$C]glucose-containing medium. Uptake was terminated by addition of 1 ml of ice-cold stopping solution (150 mmol $l^{-1}$ NaCl, 15 mmol $l^{-1}$ Mops, 1.25 mmol $l^{-1}$ KI, 1 $\mu$mol $l^{-1}$ $HgCl_2$, 100 $\mu$mol $l^{-1}$ phloretin, pH 7.5) (Weiser *et al.* 1983). Cells were rapidly washed four times with 1 ml portions of ice-cold stopping solution using an Eppendorf 5414 microcentrifuge (10 s, 12 000 *g*). After washing, the cell pellets were lysed with 0.5 ml of 0.5 % (v/v) Triton X-100 in water and 0.5 ml of 5 % (w/v) trichloroacetic acid was added. The precipitates were removed by centrifugation (12 000 *g* for 2 min) and 0.9 ml samples of protein-free supernatant counted for radioactivity by scintillation spectroscopy with appropriate quench correction. Initial rates of uptake were determined using incubation periods of between 2.5 and 10 min, depending on the hexose permeability of the cells under study. Kinetic constants were determined by nonlinear regression analysis (Enzfitter, Elsevier-Biosoft).

## Results

### *Variable 3-OMG transport in* Anguilla japonica *erythrocytes*

Representative time courses of 3-OMG uptake (5 mmol $l^{-1}$ extracellular con-

centration) by erythrocytes from two individuals, measured both in the presence and in the absence of 10 $\mu$mol $l^{-1}$ cytochalasin B, are shown in Fig. 1. Cells from both fish exhibited cytochalasin-B-sensitive and cytochalasin-B-insensitive components of 3-OMG uptake. However, there was a considerable difference in the magnitude of the former component of transport in the two animals, cells from fish A exhibiting an initial rate of cytochalasin-B-sensitive 3-OMG uptake of 6.9 mmol l cells$^{-1}$ h$^{-1}$ compared with 1.0 mmol l cells$^{-1}$ h$^{-1}$ for erythrocytes from fish B. In contrast, cytochalasin-B-insensitive 3-OMG uptake rates were similar in the two fish (0.16 and 0.15 mmol l cells$^{-1}$ h$^{-1}$ for erythrocytes from eels A and B, respectively). Intracellular [$^{14}$C]-3-OMG in cells from fish A reached a level of 2.9 mmol l cells$^{-1}$ after 2 h. This corresponds to 89 % equilibration with intracellular water, calculated on the basis that *A. japonica* erythrocytes contain 66 % (v/v) water (Fincham *et al.* 1987).

Fig. 2A shows initial rates of cytochalasin-B-sensitive 3-OMG uptake (5 mmol $l^{-1}$) measured in erythrocytes from 50 individuals. One fish (arrowed) exhibited no detectable inhibitor-sensitive sugar uptake and was defined as transport-deficient. The remaining 49 transport-positive type fish gave cytochalasin-B-sensitive 3-OMG uptake rates ranging between 0.07 and 19.5 mmol l cells$^{-1}$ h$^{-1}$. Initial rates of cytochalasin-B-insensitive 3-OMG uptake were low and much less variable (0.18–0.6 mmol l cells$^{-1}$ h$^{-1}$) (Fig. 2B). Storage of cells at 4 °C in glucose-containing medium had no measurable effect on cytochalasin-B-

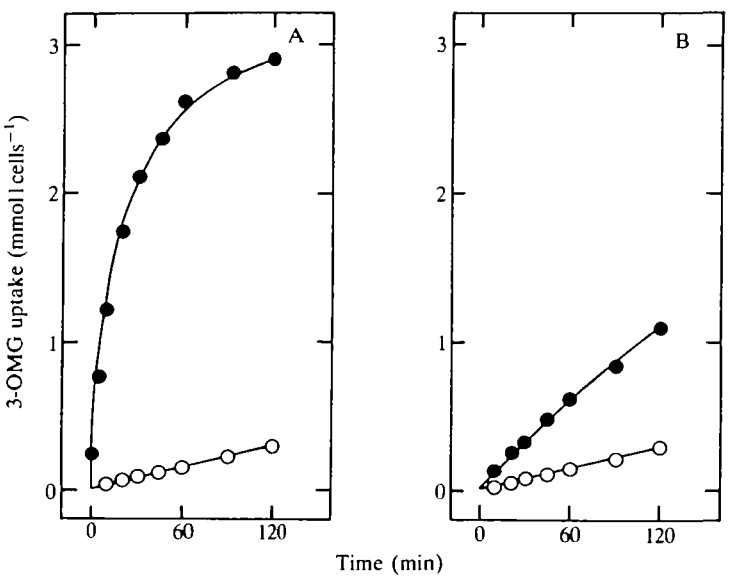


Fig. 1. Time course of 3-OMG uptake by *Anguilla japonica* erythrocytes. Uptake of 3-OMG (5 mmol $l^{-1}$ extracellular concentration, 20 °C) was measured in erythrocytes from two representative eels both in the absence (●) and in the presence (○) of 10 $\mu$mol $l^{-1}$ cytochalasin B. See Materials and methods for experimental details. Values are means of duplicate determinations.

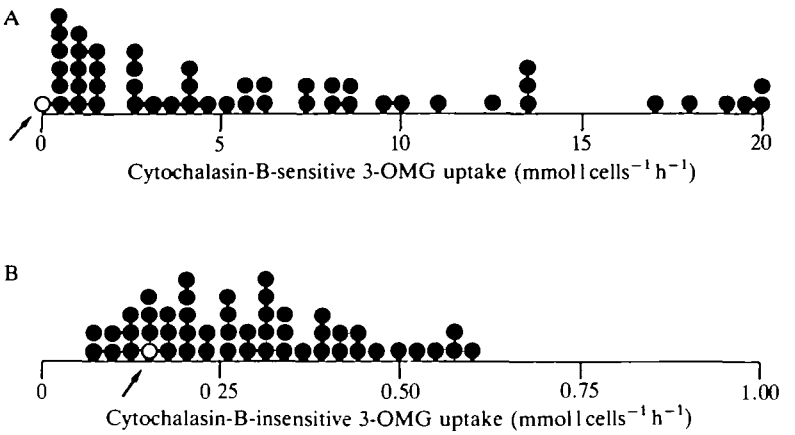


Fig. 2. Distribution of 3-OMG transport activity in *Anguilla japonica* erythrocytes. Initial rates of 3-OMG uptake (5 mmol $l^{-1}$) were determined in the absence and in the presence of 10 $\mu$mol $l^{-1}$ cytochalasin B, as described in Materials and methods. (A) The distribution of cytochalasin-B-sensitive 3-OMG uptake activity. (B) The corresponding distribution for the cytochalasin-B-insensitive component of uptake. Each point represents data from a different fish. Erythrocytes from one eel (○, arrowed) were totally deficient in cytochalasin-B-sensitive 3-OMG transport activity.

sensitive 3-OMG uptake. For example, cells from one fish gave a cytochalasin-B-sensitive uptake rate of 0.86 mmol l cells$^{-1}$ h$^{-1}$ immediately after blood collection (5 mmol $l^{-1}$ 3-OMG) and rates of 0.86, 0.88 and 0.85 mmol l cells$^{-1}$ h$^{-1}$ after 24, 48 and 72 h of storage, respectively.

Of 20 eels tested, intracellular ATP levels ranged from 1.7 to 5.2 mmol l cells$^{-1}$. Cytochalasin-B-sensitive 3-OMG uptake rates in erythrocytes from these fish varied between 0.12 and 17.2 mmol l cells$^{-1}$ h$^{-1}$. There was no correlation between the two parameters.

### *Kinetic and inhibitor studies of 3-OMG transport in* Anguilla japonica *erythrocytes*

Fig. 3 shows a typical concentration-dependence curve for 3-OMG uptake by eel erythrocytes at 20°C measured over the concentration range 0.5–10 mmol $l^{-1}$ in the presence and in the absence of 10 $\mu$mol $l^{-1}$ cytochalasin B. The cytochalasin-B-sensitive component of 3-OMG uptake was saturable and conformed to simple Michaelis–Menten kinetics. Kinetic constants (±S.E.) estimated by nonlinear regression analysis were: $K_m$ 1.22±0.32 mmol $l^{-1}$ and $V_m$ 8.41±0.59 mmol l cells$^{-1}$ h$^{-1}$. Cytochalasin-B-insensitive 3-OMG uptake was linear over the same concentration range.

To investigate the substrate specificity of the cytochalasin-B-sensitive saturable component of 3-OMG uptake in eel erythrocytes, various D- and L-hexoses were tested as inhibitors of 3-OMG transport (Table 1). Of the sugars tested, D-glucose, D-galactose and D-deoxyglucose were found to be effective inhibitors of cytochal-

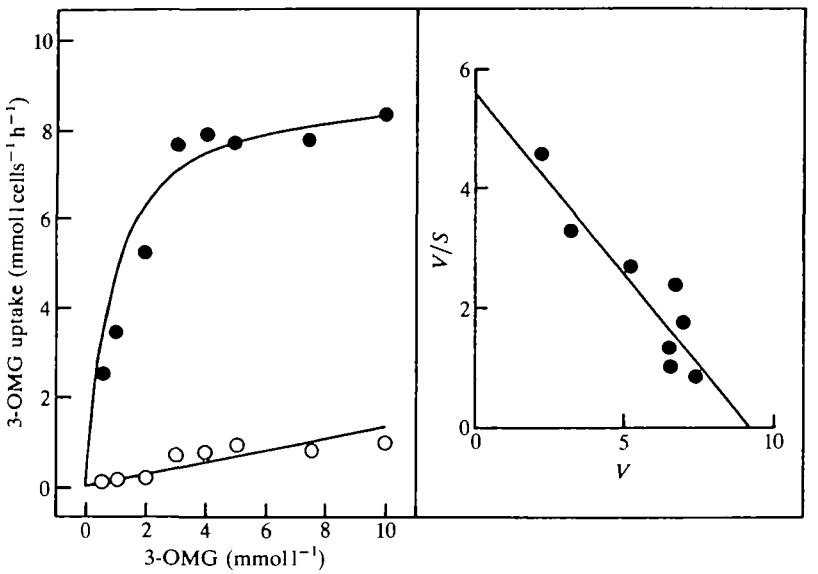


Fig. 3. Concentration dependence of 3-OMG uptake by *Anguilla japonica* erythrocytes. Initial rates of 3-OMG uptake (0.5–10 mmol l$^{-1}$) were measured in the presence (○) and in the absence (●) of 10 μmol l$^{-1}$ cytochalasin B. Apparent $K_m$ and $V_m$ values for the cytochalasin-B-sensitive component of transport, shown as an Eadie–Hofstee plot, are given in the text.

Table 1. *Inhibition of 3-OMG uptake by other hexoses*

| | Cytochalasin-B-sensitive 3-OMG uptake (% of control) |
|---|---|
| No competitor | 100 |
| D-Glucose (5 mmol l$^{-1}$) | 50 |
| (10 mmol l$^{-1}$) | 34 |
| D-Fructose (5 mmol l$^{-1}$) | 102 |
| D-Galactose (5 mmol l$^{-1}$) | 89 |
| (10 mmol l$^{-1}$) | 68 |
| D-Deoxyglucose (5 mmol l$^{-1}$) | 39 |
| (10 mmol l$^{-1}$) | 22 |
| L-Glucose (10 mmol l$^{-1}$) | 102 |

Various D- and L-hexoses were tested as inhibitors of cytochalasin-B-sensitive 3-OMG transport in *Anguilla japonica* erythrocytes at 20°C. Initial 3-OMG uptake rates (±10 μmol l$^{-1}$ cytochalasin B) were measured at 5 mmol l$^{-1}$ extracellular concentration in the absence and in the presence of competing sugar (5 or 10 mmol l$^{-1}$) added simultaneously. None of the compounds tested had a significant effect on cytochalasin-B-insensitive 3-OMG uptake.

Values are means of duplicate estimates.

The control cytochalasin-B-sensitive uptake rate in the cells used in this experiment was 17.2 mmol l cells$^{-1}$ h$^{-1}$.

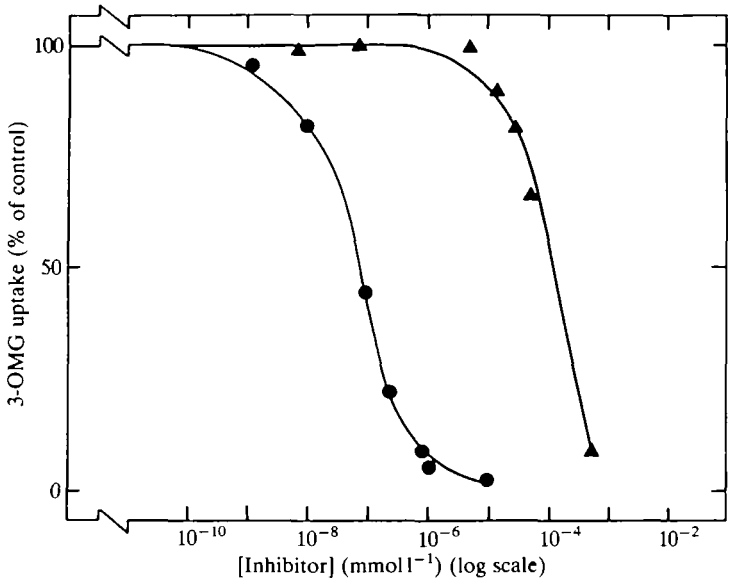


Fig. 4. Inhibition of 3-OMG uptake in *Anguilla japonica* erythrocytes by phloretin and cytochalasin B. Erythrocytes were preincubated in the absence and in the presence of phloretin (▲) or cytochalasin B (●) for 30 min at 20°C. Initial rates of 3-OMG influx (5 mmol $l^{-1}$) were then measured as described in Materials and methods and corrected for the cytochalasin-B-insensitive component of uptake (determined in the presence of 10 $\mu$mol $l^{-1}$ cytochalasin B). Values are the means of duplicate estimates. Control cytochalasin-B-sensitive 3-OMG uptake rates were 1.6 and 1.2 mmol l cells$^{-1}$ $h^{-1}$ for the cytochalasin B and phloretin inhibition experiments, respectively. Phloretin (0.5 mmol $l^{-1}$) had no effect on cytochalasin-B-insensitive 3-OMG transport.

asin-B-sensitive 3-OMG uptake and inhibition was concentration dependent. The degree of inhibition was in the order D-deoxyglucose>D-glucose>D-galactose and is consistent with the relative affinities of these sugars for the human erythrocyte sugar transport system (Naftalin and Holman, 1977). L-Glucose, which is not a substrate for the mammalian hexose transport system, had no detectable effect on 3-OMG uptake in eel erythrocytes.

In addition to cytochalasin B, phloretin also inhibits sugar transport in a number of cell types. Fig. 4 shows concentration dependence curves for these two compounds as inhibitors of saturable (cytochalasin-B-sensitive) 3-OMG uptake in eel erythrocytes. $IC_{50}$ values were 0.125 and 80 $\mu$mol $l^{-1}$ (5 mmol $l^{-1}$ extracellular 3-OMG) for cytochalasin B and phloretin, respectively. To estimate apparent $K_i$, the results were fitted by least-squares analysis to the equation:

$$V = V_o K_i/(K_i + I),$$

where $V_o$ is the inhibitor-sensitive rate, I, the inhibitor concentration and $K_i$, the inhibition constant (Simons, 1983*a*). The inhibition constant obtained for cytochalasin B (0.10±0.01 $\mu$mol $l^{-1}$) compares favourably with an estimate of 0.1–0.2 $\mu$mol $l^{-1}$ for the apparent $K_d$ of cytochalasin B binding to the human

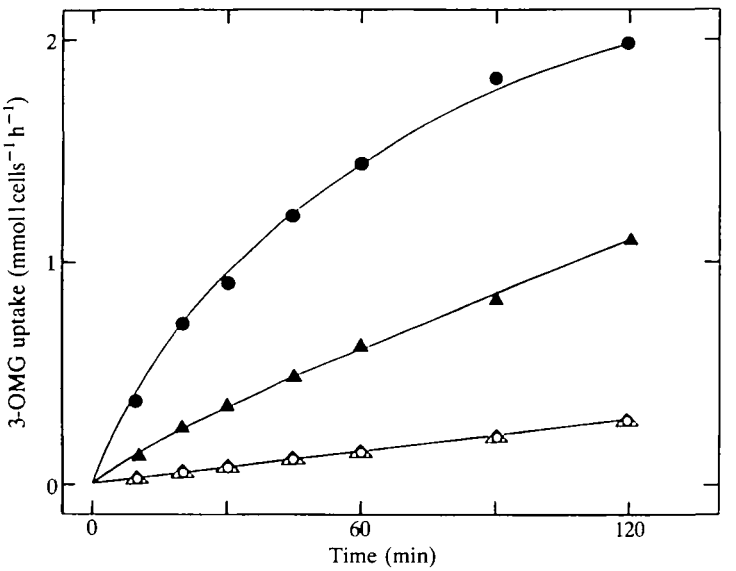


Fig. 5. Effect of noradrenaline on the time-course of 3-OMG transport in *Anguilla japonica* erythrocytes. Uptake of 3-OMG was measured at 5 mmol $l^{-1}$ extracellular concentration in the presence (open symbols) and in the absence (closed symbols) of 10 $\mu$mol $l^{-1}$ cytochalasin B; (▲, △) control erythrocytes preincubated for 2 h at 20°C in the absence of catecholamine; (●, ○) cells preincubated in the presence of 100 $\mu$mol $l^{-1}$ noradrenaline.

erythrocyte glucose transporter (Jung and Rampal, 1977). In contrast, the phloretin inhibition constant of 48±4 $\mu$mol $l^{-1}$ is substantially higher than that for inhibition of glucose transport in human erythrocytes (0.5–2.5 $\mu$mol $l^{-1}$) (Krupka, 1985). Similar high apparent $K_i$ values (28–43 $\mu$mol $l^{-1}$) have been reported for phloretin inhibition of sugar transport in avian erythrocytes (Simons, 1983*a*).

*Effects of catecholamines on 3-OMG transport in* Anguilla japonica *erythrocytes*

As in avian erythrocytes, 3-OMG uptake by eel erythrocytes was increased by catecholamines. Fig. 5 shows typical time courses of 3-OMG uptake in noradrenaline-treated (100 $\mu$mol $l^{-1}$, 2 h preincubation) and control (untreated) erythrocytes measured both in the absence and in the presence of 10 $\mu$mol $l^{-1}$ cytochalasin B. The cytochalasin-B-sensitive 3-OMG uptake rate increased from 0.7 to 2.2 mmol l cells$^{-1}$ h$^{-1}$, while cytochalasin-B-insensitive uptake of 3-OMG remained unchanged.

Catecholamine stimulation of cytochalasin-B-sensitive 3-OMG transport depended both upon the period of exposure to catecholamine and upon the catecholamine concentration. Noradrenaline and adrenaline were equally effective, maximum stimulation occurring after 2 h of exposure (Fig. 6) and at catecholamine concentrations in the range 50–100 $\mu$mol $l^{-1}$ (Fig. 7). There was no detectable stimulation of 3-OMG transport at concentrations below 0.01 $\mu$mol $l^{-1}$

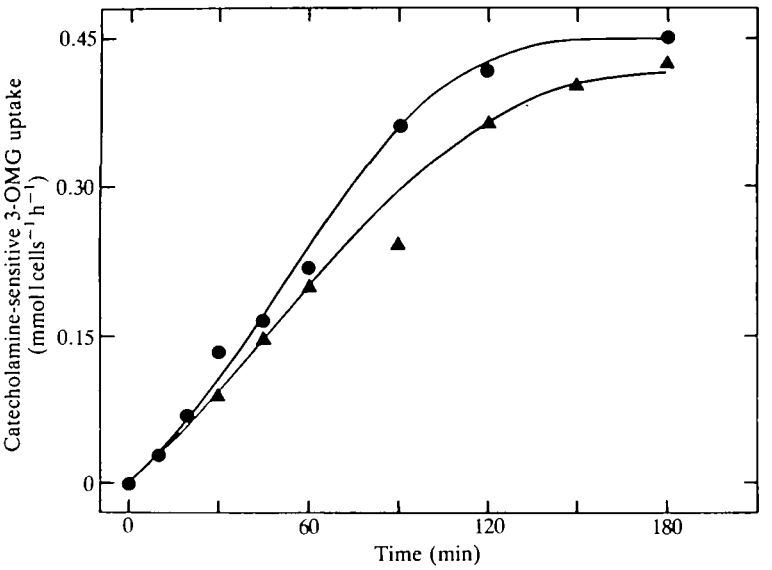


Fig. 6. Effect of preincubation time on the magnitude of catecholamine-induced 3-OMG transport in *Anguilla japonica* erythrocytes. Erythrocytes were preincubated at 20°C with 100 $\mu$mol l$^{-1}$ of either noradrenaline (●) or adrenaline (▲) for varying lengths of time. After incubation, initial rates of cytochalasin-B-sensitive 3-OMG uptake (5 mmol l$^{-1}$) were measured as described in the text. Catecholamine-induced 3-OMG fluxes were calculated as the difference in uptake rate measured in control cells incubated in the absence of hormone and cells incubated in the presence of adrenaline or noradrenaline.

and half-maximal stimulation occurred at approximately 1 $\mu$mol l$^{-1}$. This latter catecholamine concentration is similar to that required for half-maximal stimulation of 3-OMG uptake into goose erythrocytes (Whitfield *et al.* 1974). For transport-positive type eels with basal 3-OMG (5 mmol l$^{-1}$) permeabilities in the range 0.24–3.13 mmol l cells$^{-1}$ h$^{-1}$, maximal stimulation of sugar transport activity appeared to be independent of the starting cytochalasin-B-sensitive 3-OMG permeability of the cells (Table 2). Quantitatively similar catecholamine-induced increases in 3-OMG permeability were also detected in cells with higher basal transport activities, but this was not verified statistically. The fish previously identified as 3-OMG transport-deficient (Fig. 2) failed to respond to catecholamine stimulation (see also Table 2).

Fig. 8 compares the concentration dependence of 3-OMG uptake (range 0.05–5 mmol l$^{-1}$) in control transport-positive cells and cells preincubated with noradrenaline. Kinetic constants for cytochalasin-B-sensitive 3-OMG uptake were: $K_m$ 1.38±0.27 and 1.47±0.31 mmol l$^{-1}$ and $V_m$ 2.52±0.18 and 1.01±0.08 mmol l cells$^{-1}$ h$^{-1}$ for noradrenaline-treated and control cells, respectively. In agreement with the results presented in Fig. 5, noradrenaline had no effect on cytochalasin-B-insensitive 3-OMG uptake.

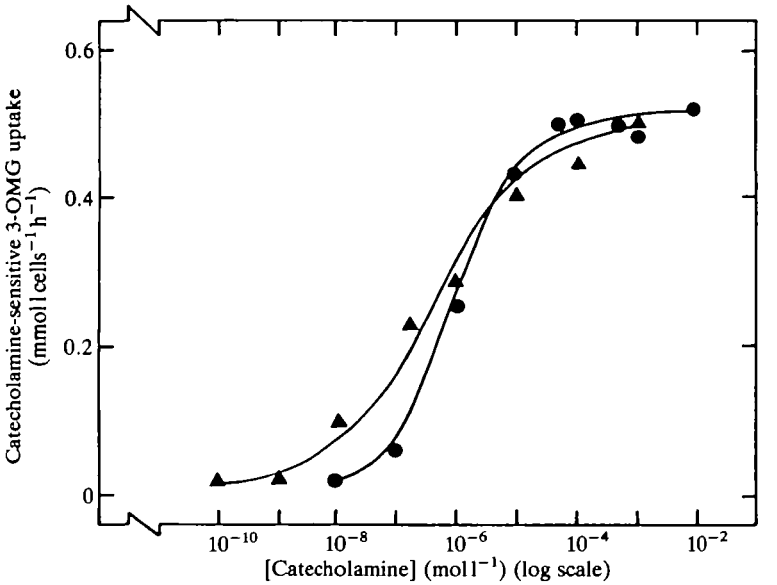


Fig. 7. Concentration dependence of catecholamine stimulation of 3-OMG transport in *Anguilla japonica* erythrocytes. Cells were preincubated with varying concentrations ($10^{-10}$–$10^{-2}$ mol l$^{-1}$) of adrenaline (▲) or noradrenaline (●) for 2 h at 20°C. Initial rates of cytochalasin-B-sensitive 3-OMG transport (5 mmol l$^{-1}$) were then measured as described in Materials and methods. Catecholamine-induced 3-OMG fluxes were calculated as the difference in uptake rate measured in control cells incubated in the absence of hormone and cells incubated in the presence of adrenaline or noradrenaline.

### *Uptake of* D- *and* L-*glucose by* Anguilla japonica *erythrocytes*

Table 3 shows that, in addition to cytochalasin-B-sensitive 3-OMG uptake, eel erythrocytes exhibited cytochalasin-B-sensitive, stereospecific transport of glucose. The observed cytochalasin-B-sensitive D-glucose uptake rate (5.9±0.1 mmol l cells$^{-1}$ h$^{-1}$ at 5 mmol l$^{-1}$ extracellular D-glucose) was similar to that for 3-OMG uptake in the same cells (6.9±0.1 mmol l cells$^{-1}$ h$^{-1}$) and was equivalent to the stereospecific glucose uptake rate of 5.9±0.1 mmol l cells$^{-1}$ h$^{-1}$, defined as the difference in permeability between D- and L-glucose. L-Glucose uptake was not inhibited by cytochalasin B (0.09±0.01 and 0.08±0.01 mmol l cells$^{-1}$ h$^{-1}$ in the absence and in the presence of cytochalasin B, respectively). These L-glucose uptake rates are approximately half that for cytochalasin-B-insensitive uptake of D-glucose. In other words, cytochalasin-B-insensitive glucose uptake was partially stereospecific. A similar observation has been made with respect to D- and L-glucose uptake in cytochalasin-B-treated pigeon erythrocytes (Simons, 1983*a*).

In the experiment presented in Table 3, cytochalasin-B-insensitive uptake of 3-OMG was substantially greater than the corresponding fluxes for D- and L-glucose. This difference is a consequence of the greater hydrophobicity of

Table 2. *Catecholamine-stimulation of 3-OMG transport in* Anguilla japonica *erythrocytes*

| Fish | Treatment | Cytochalasin-B-sensitive 3-OMG uptake (mmol l cells$^{-1}$ h$^{-1}$) | Catecholamine-induced cytochalasin-B-sensitive 3-OMG uptake (mmol l cells$^{-1}$ h$^{-1}$) |
|---|---|---|---|
| 1 | Control | 0 | 0 |
| | Adrenaline | 0 | 0 |
| | Noradrenaline | 0 | 0 |
| 2 | Control | 0.24 | |
| | Adrenaline | 0.65 | 0.41 |
| | Noradrenaline | 0.62 | 0.38 |
| 3 | Control | 0.71 | |
| | Adrenaline | 1.14 | 0.43 |
| | Noradrenaline | 1.12 | 0.41 |
| 4 | Control | 3.13 | |
| | Adrenaline | 3.60 | 0.47 |
| | Noradrenaline | 3.84 | 0.71 |

This table summarizes a series of catecholamine-stimulation experiments from four individual fish, each exhibiting different basal cytochalasin-B-sensitive 3-OMG transport activities. Cells were incubated either in the absence or in the presence of adrenaline or noradrenaline (both at 100 $\mu$mol l$^{-1}$) for 2 h before assay of initial rates of cytochalasin-B-sensitive 3-OMG transport at an extracellular concentration of 5 mmol l$^{-1}$.

Results are means of duplicate estimates.

3-OMG, allowing it to diffuse through the lipid bilayer more rapidly (Hillman *et al.* 1959).

### *Sugar uptake in* Monopterus albus *and* Salmo gairdneri *erythrocytes*

Erythrocytes from individual *M. albus* and *S. gairdneri* exhibited no detectable cytochalasin-B-sensitive 3-OMG uptake either in the absence or in the presence of 100 $\mu$mol l$^{-1}$ catecholamines. Similarly, D-glucose uptake by erythrocytes from *M. albus* was not inhibited by cytochalasin B. Mean results for a number of fish of each species are presented in Table 4. In agreement with the data for cytochalasin-B-insensitive uptake of D- and L-glucose in erythrocytes from *A. japonica* (Table 3), D-glucose uptake rates in cells from *M. albus* were greater than for L-glucose, although the absolute magnitudes of the fluxes were smaller. This observation suggests that the *A. japonica* result was not because of incomplete inhibition of saturable D-glucose uptake by cytochalasin B.

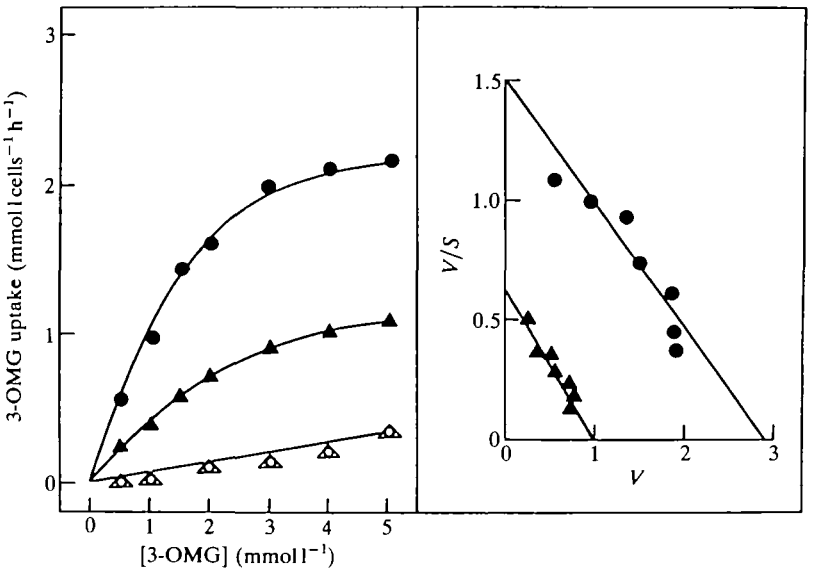


Fig. 8. Concentration dependence of 3-OMG uptake by noradrenaline-treated *Anguilla japonica* erythrocytes. Cells were incubated for 2 h at 20°C in the absence and in the presence of 100 $\mu$mol l$^{-1}$ noradrenaline. Initial rates of 3-OMG uptake (0.5–5 mmol l$^{-1}$) were then measured as described in text. (▲) control erythrocytes, (●) noradrenaline-treated erythrocytes, (△) control erythrocytes+10 $\mu$mol l$^{-1}$ cytochalasin B, (○) noradrenaline-treated erythrocytes+10 $\mu$mol l$^{-1}$ cytochalasin B. Kinetic constants for the cytochalasin-B-sensitive components of transport, shown as Eadie–Hofstee plots, are given in the text.

Table 3. *D- and L-glucose uptake by* Anguilla japonica *erythrocytes*

| | Uptake rate (mmol l cells$^{-1}$ h$^{-1}$) | | |
|---|---|---|---|
| | − cytochalasin B | + cytochalasin B | Difference |
| 3-OMG | 7.70±0.10 | 0.80±0.01 | 6.90±0.10 |
| D-Glucose | 6.00±0.10 | 0.15±0.02 | 5.85±0.10 |
| L-Glucose | 0.09±0.01 | 0.08±0.01 | 0.01±0.02 |

Initial rates of 3-OMG, D- or L-glucose uptake (5 mmol l$^{-1}$ extracellular concentration) were measured in the absence and in the presence of 10 $\mu$mol l$^{-1}$ cytochalasin B.

Values (±S.E.) are means of triplicate estimates for cells from the same fish. The column labelled 'Difference' represents the difference in the uptake rate measured in the presence and in the absence of cytochalasin B.

## Discussion

Of the three teleost species examined, erythrocytes from both the paddyfield eel (*M. albus*) and the rainbow trout (*S. gairdneri*) exhibited uniformly low rates of 3-OMG uptake in the range 0.10–0.15 mmol l cells$^{-1}$ h$^{-1}$ (5 mmol l$^{-1}$ extracellular 3-OMG, 20°C). D-Glucose uptake rates in *M. albus* were lower, with a mean value

Table 4. *Sugar uptake in erythrocytes from* Monopterus albus *and* Salmo gairdneri

| | Uptake rate (mmol l cells$^{-1}$ h$^{-1}$) | | |
|---|---|---|---|
| | − cytochalasin B | + cytochalasin B | Difference |
| *M. albus* | | | |
| 3-OMG | | | |
| Control ($N$=10) | 0.140±0.015 | 0.131±0.035 | 0.009±0.04 |
| Adrenaline-treated erythrocytes ($N$=10) | 0.130±0.013 | 0.128±0.005 | 0.002±0.014 |
| Noradrenaline-treated erythrocytes ($N$=10) | 0.146±0.013 | 0.143±0.010 | 0.003±0.016 |
| Glucose | | | |
| D-Glucose ($N$=7) | 0.052±0.009 | 0.042±0.004 | 0.010±0.010 |
| L-Glucose ($N$=7) | 0.021±0.005 | 0.020±0.006 | 0.001±0.008 |
| *S. gairdneri* | | | |
| 3-OMG | | | |
| Control ($N$=5) | 0.120±0.01 | 0.121±0.011 | 0 |
| Adrenaline-treated erythrocytes ($N$=5) | 0.125±0.02 | 0.123±0.010 | 0.002±0.022 |
| Noradrenaline-treated erythrocytes ($N$=5) | 0.114±0.01 | 0.114±0.020 | 0 |

Uptake of 3-OMG, D- and L-glucose were measured at 5 mmol l$^{-1}$ extracellular concentration in the presence and in the absence of 10 μmol l$^{-1}$ cytochalasin B.

For catecholamine-treated cells, erythrocytes were preincubated with 100 μmol l$^{-1}$ noradrenaline or adrenaline for 2 h.

Values (±S.E.) are means of triplicate estimates for erythrocytes from $N$ fish.

'Difference' represents the cytochalasin-B-sensitive uptake rate.

of approximately 0.05 mmol l cells$^{-1}$ h$^{-1}$ at 5 mmol l$^{-1}$ extracellular D-glucose. Sugar uptake in erythrocytes from these two species was not inhibited by cytochalasin B and was insensitive to catecholamine stimulation. Erythrocytes from one common eel (*A. japonica*) were also transport-deficient with respect to cytochalasin-B-sensitive 3-OMG uptake. In marked contrast, erythrocytes from the other common eels exhibited significant and highly variable rates of cytochalasin-B-sensitive 3-OMG transport in the range 0.07–19.5 mmol l cells$^{-1}$ h$^{-1}$ when assayed under identical conditions. This variability was not seasonal, did not depend upon the time the animals had been retained in aquaria before bleeding or upon eel size and was not correlated with intracellular ATP levels.

The stress hormones adrenaline and noradrenaline are released into the fish bloodstream during handling and bleeding and have been shown to stimulate erythrocyte ion transport and cause cell swelling (Bourne and Cossins, 1982; DeVries and Ellory, 1982; Baroin *et al.* 1984). A similar phenomenon occurs in avian species (Kregenow, 1975) and catecholamines have been shown to increase cytochalasin-B-sensitive sugar transport in both pigeon and goose erythrocytes (Whitfield and Morgan, 1973; Whitfield *et al.* 1974; Bihler *et al.* 1982; Simons,

1983*a*). Several factors indicate that catecholamine effects cannot account for the variation in *A. japonica* 3-OMG uptake rates seen in the present study. First, erythrocytes were washed free of plasma and routinely stored overnight in glucose-containing medium at 4°C before assay of transport activity (see Materials and methods). In addition, although the cells were sensitive to catecholamine stimulation, the magnitude of the catecholamine-induced increase in 3-OMG transport activity appeared to be independent of the basal cytochalasin-B-sensitive 3-OMG permeability of the cells (Table 2). Also, cells from one common eel were totally deficient with respect to cytochalasin-B-sensitive transport activity and did not respond to catecholamine stimulation.

It is therefore likely that glucose transport variation in *A. japonica* erythrocytes is under genetic control. This species therefore represents an intermediate situation between the hagfish, whose erythrocytes exhibit a high cytochalasin-B-sensitive sugar permeability (Ingermann *et al.* 1984), and *M. albus* and rainbow trout, whose erythrocytes are devoid of cytochalasin-B-sensitive transport activity. Erythrocytes from the brown trout are also likely to be transport-deficient (Bolis *et al.* 1971). Genetically determined transport polymorphisms have been identified in erythrocytes from a number of mammalian species. These include amino acid transport in sheep and horse erythrocytes (Young *et al.* 1975; Fincham *et al.* 1985), cation transport in sheep, goat, cattle, buffalo and dog erythrocytes (Ellory and Tucker, 1983) and nucleoside transport in sheep erythrocytes (Tucker and Young, 1988).

Cytochalasin-B-sensitive 3-OMG transport in *A. japonica* erythrocytes was shown to be saturable (apparent $K_m$ 1.2–1.5 mmol $l^{-1}$) and to be inhibited by other D-hexoses (but not by L-glucose) and by phloretin. In these respects the system resembles sugar transporters in both avian and mammalian erythrocytes. Direct transport experiments with D- and L-glucose provided further evidence of stereospecificity. As shown in Figs 3 and 8, transport variation among erythrocytes from different fish was a consequence of differences in $V_m$ rather than $K_m$ and presumably reflects differences in the cellular density of glucose transporter polypeptides. As in avian erythrocytes, the transporter was stimulated by catecholamines. Kinetically, catecholamines increased the $V_m$ of transport without changing apparent $K_m$. The mechanism of transport stimulation by catecholamines remains to be investigated. Whitfield *et al.* (1974) reported that catecholamine stimulation of sugar transport in avian erythrocytes is associated with a lowering of intracellular ATP level. This effect is not mediated through $\alpha$- or $\beta$-adrenergic receptors. Stimulation of sugar transport in avian erythrocytes can also be brought about by metabolic depletion (Whitfield and Morgan, 1973; Bihler *et al.* 1982, 1985; Simons, 1983*a*,*b*). Increased sugar transport activity under these conditions is unlikely to involve changes in cytosolic $Ca^{2+}$ concentration (Simons, 1983*b*). ATP directly modulates glucose transport in human erythrocytes (Carruthers, 1986*a*,*b*).

Physiological catecholamine concentrations in fish plasma may rise to 0.5–1.5 $\mu$mol $l^{-1}$ during stress (Mazeaud and Mazeaud, 1981; Bourne and Cossins

1982). Such catecholamine levels would be sufficient to induce a half-maximal stimulation of glucose transport activity in *A. japonica* erythrocytes (Fig. 7), raising the possibility that erythrocyte glucose permeability may increase *in vivo* in response to prolonged stress. In contrast to trout cells (Bourne and Cossins, 1982), *A. japonica* erythrocytes do not swell in the presence of adrenaline or noradrenaline (D. A. Fincham and J. D. Young, unpublished observation). Hormone-induced changes in cytochalasin-B-sensitive glucose transport activity are not, therefore, a secondary consequence of cell volume changes.

Cytochalasin-B-insensitive sugar transport in fish (and avian) erythrocytes is partially stereospecific (Tables 3 and 4). It has been suggested that this phenomenon in avian cells reflects stereoselective diffusion of glucose across the lipid bilayer (Simons, 1983*a*). However, D- and L-glucose exhibit equivalent permeability coefficients in phospholipid vesicles (C. M. Tse and J. D. Young, unpublished observation). Similarly, pig erythrocytes exhibit equivalent rates for cytochalasin-B-insensitive transport of D- and L-glucose (Young *et al.* 1985). An alternative possibility is that a component of this flux in fish and avian erythrocytes represents stereospecific mediated transport *via* a minor cytochalasin-B-insensitive transport system. Cytochalasin-B-insensitive sugar transport has been observed in rabbit erythrocytes (Albert, 1984). Rates of cytochalasin-B-insensitive uptake of 3-OMG and D- and L-glucose in *A. japonica* erythrocytes were significantly higher than the corresponding fluxes in *M. albus* and the rainbow trout.

For *M. albus*, measured rates of D-glucose uptake were similar to those reported previously by us for mature pig erythrocytes (Young *et al.* 1985, 1986). For pig erythrocytes, this low glucose permeability is insufficient to maintain normal ATP:ADP ratios (Kim and McManus, 1971; Young *et al.* 1985). In contrast to mammalian erythrocytes, and by analogy with avian erythrocytes (Kregenow, 1975), fish erythrocytes have the potential to metabolize glucose *via* the tricarboxylic acid cycle (Greaney and Powers, 1978; Tetens and Lykkeboe, 1981). However, additional metabolic substrates might still be required. One potential candidate is inosine, the physiological energy source used by pig erythrocytes (Young *et al.* 1985, 1986). In preliminary experiments we have established that erythrocytes from both the common eel and *M. albus* are capable of rapid nitrobenzylthioinosine (NBMPR)-sensitive nucleoside transport (approximately $2\times10^5$ nucleoside transporters per cell as determined by [$^3$H]NBMPR binding).

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