Hexokinase activities from both tissues were slightly unaffected by fasting (Fig. 1) but not affected by glibenclamide treatment in any of the experiments (Figs 1–3). The inductive effect of glibenclamide treatment on glucose metabolism by glibenclamide stimulation. This is proven by the fact that fed rats made diabetic (serum glucose concentrations above 15 mM) by injection of alloxan (100 mg/kg b. wt. four days before the experiment) presented liver glucokinase activities in the range of those observed in the liver from fasted non-diabetic rats [14] (4.7 ± 0.4 mU/mg protein; N = 6). These low liver glucokinase activities from diabetic rats were not significantly raised by glibenclamide treatment (6.1 ± 0.1 mU/mg protein N = 4) but normalized by insulin treatment (12.4 ± 1.3 mU/mg protein; N = 6).

Thus our experiments have shown that hypoglycemic sulfonylureas such as glibenclamide can maintain their long-term hypoglycemic action through induction of pancreatic islet and liver glucokinase by insulin. Induction of pancreatic islet glucokinase keeps the glucose recognition system sensitive for the initiation of insulin secretion and biosynthesis by glucose stimulation. This induction of pancreatic islet glucokinase by insulin augments the signal generating flux rate through the glycolytic pathway relative to the actual glucose concentration surrounding the pancreatic B-cell. A sustained insulin secretory response of the pancreatic B-cell to absorptive and post-absorptive glucose keeps liver glucokinase in an induced state. This enables the liver to regulate the blood glucose concentration. Thus the induction of glucokinase in vivo represents a link between the pancreatic and extrapancreatic effects of glibenclamide and provides an explanation for the interaction between pancreatic islets and liver in the maintenance of glucose homeostasis [15].

Acknowledgements—S.L. is greatly indebted to Professor D. G. Walker, Dept. of Biochemistry, University of Birmingham, for providing glucokinase antibody. This investigation was supported by the Deutsche Forschungsgemeinschaft.

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REFERENCES


Uptake and accumulation of gentamicin in the developing inner ear of the mouse
in vitro

(Received 18 November 1985; accepted 24 January 1986)

The sequences of both the bactericidal and the nephrotoxic actions of the aminoglycoside antibiotics include an energy-dependent uptake into the affected cells [1–3]. Whether an analogous step is also part of the otoxic mechanism is not known. Pharmacokinetic studies relating to the cochlear actions of the aminoglycosides have only provided information on drug disposition in the fluids [4, 5] and the tissues of the inner ear [6, 7]. Questions of transport mechanisms or related issues such as whether the aminoglycosides enter the cells passively along, or actively against, a concentration gradient have not been addressed yet. Electrophysiological studies, however, have suggested that the otoxic actions of gentamicin require an as yet undefined energy-dependent process [8], and a cellular uptake was postulated in a recent model of aminoglycoside otoxicity [9]. To determine parameters of uptake, the drug con-
were removed, and their developmental stages were assessed [12]. The embryonic inner ear is easy to manipulate, will differentiate in culture [10], and is susceptible to aminoglycosides [11]. Drug concentrations in the culture medium can be defined and compared to those in the otic explants.

Materials and methods

Organ culture. Normal hybrid CBA/J and C57 mouse embryos were obtained by mating CBA-J and C57 mice (Jackson Laboratories, Bar Harbor, ME). The day at which a mucoid vaginal plug was found was designated as the first day of gestation. On gestational day 16, pregnant mice were killed by cervical dislocation, and their gravid uteri were placed in Dulbecco's phosphate-buffered saline (PBS). Embryos were removed, and their developmental stages were assessed [12]. The embryonic inner ear with its associated cartilaginous otic capsule and ganglionic tissue was dissected and explanted to organ culture dishes. Care was taken to free the otic explants of any adhering middle ear mesenchyme.

Each culture dish (Falcon, 35 × 10 mm; Becton, Dickinson & Co., Oxnard, CA) contained three otic explants in 2 ml of Neumann-Tytell medium (Gibco Laboratories, Grand Island, NY), supplemented with 20% fetal calf serum and 25 μg ascorbic acid/ml. Explants were grown under 5% CO₂ in air (95% relative humidity) at 36.5°C for 8 days. The radioimmunoassay (American Bioclinical, Portland, OR) was modified after Meulemans et al. [14] to increase sensitivity.

Results and discussion

Otic explants developed in organ culture without any gross pathology induced by gentamicin. The average gain in weight and protein content of the explants during culture was not affected significantly by the presence of the drug (13.5 μg increase in protein/explant from day 1 to day 5 for controls vs 12.7 μg at 10 μg gentamicin/ml of medium). Morphogenesis appeared normal with well-defined semicircular ducts (SCD) and a coiled cochlear duct (CD) (Fig. 1). Incubations without gentamicin served as controls ("C", control incubation without gentamicin). The significant finding of this study is the observation that otic explants accumulated gentamicin over concentrations in the culture medium. Concentrations of gentamicin in the inner ear were determined assuming that 1 mg (wet weight) of tissue is equivalent to 1 μl of volume. Over the range of 0.1 to 17 μg drug/ml of medium, the ratios of concentrations in the explants to those in the medium were inversely proportional to the drug concentration. Genta-
Biochemical Pharmacology, incubated for 3 and 5 days at 0.19 μg gentamicin/ml

Methods. Both sets of experiments were incorporated into this table. In the first set, three explants were incubated experiments and analyzed as described in Materials and medium. Otic explants were analyzed individually for their metabolism together at the end of the incubation. To obtain gentamicin effective when given orally [2,4]. It does not induce its own quantity to achieve therapeutic blood levels. Thus, it is weight.

concentrations/mg explant, total gentamicin for these explants was calculated and divided by their combined weight.

Numbers are means of at least duplicate determinations between the beginning and the end of the incubation. To obtain gentamicin concentrations/mg protein and standard deviations of the explants that were cultured together per dish were weighed together at the end of the incubation.

Table 1. Accumulation of gentamicin in the inner ear

<table>
<thead>
<tr>
<th>Incubation time (days)</th>
<th>Gentamicin concentration (μg/ml)</th>
<th>Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.12</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>3</td>
<td>2.10</td>
<td>2.10</td>
</tr>
<tr>
<td>4</td>
<td>2.20</td>
<td>2.20</td>
</tr>
<tr>
<td>5</td>
<td>2.30</td>
<td>2.30</td>
</tr>
</tbody>
</table>

* Concentration at the end of the culture period. There were no significant differences in drug concentrations between the beginning and the end of the incubation. Numbers are means of at least duplicate determinations (each within 10% of the mean).

† Ratio of gentamicin concentrations in otic explants to those in the culture medium.

micin concentrations were up to 16-fold higher in the otic explants (Table 1).

The results clearly indicate that the uptake of gentamicin into the inner ear proceeds against a concentration gradient with an apparent Km of approximately 1.7 μM. Inner ears of CBA/C57 hybrid mouse embryos were explanted at gestational day 16 and maintained in organ culture for 1, 3 or 5 days in media containing from 0 to 17 μg gentamicin/ml. Gentamicin concentrations in the otocysts increased with incubation time and exceeded the drug concentration in the medium up to 16-fold. This is the first suggestion that tissues of the inner ear may actively transport aminoglycoside antibiotics by a high-affinity uptake system.

Acknowledgements—The authors are indebted to Laila Mahran and Mary Harrington for excellent assistance. This work was supported by Research Grant NS-13792 and NS-08365 from the National Institutes of Health.

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Ketoconazole hepatotoxicity: an in vitro model

(Received 7 February 1985; accepted 24 January 1986)

Ketoconazole is the newest in a series of imidazole-derivative antifungal agents. It has a broad spectrum of activity against both superficial and systemic mycoses [1–3]. Ketoconazole is distinct from other antifungal imidazoles in clinical use. It is the only one that is absorbed in sufficient amount to achieve therapeutic blood levels. Thus, it is effective when given orally [2, 4]. It does not induce its own metabolism in vivo, nor that of other drugs in vitro [5].

Like other imidazoles, however, it inhibits the metabolism of some drugs in clinical situations [6, 7]; recent reports have suggested that it does so by inhibiting the microsomal P-450 enzyme system [8].

As clinical use of ketoconazole increased, reports of adverse hepatic reactions appeared [9–11]. There are two general categories of reactions. Asymptomatic elevations of hepatocellular enzymes occur in 5–10% of patients at...