EFFECTS OF SOME DRUGS ON ISLET FUNCTION IN CATFISH

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Summary : Blood glucose variations and concomitant bioptical cytopathological changes in the pancreatic islets following treatment with certain drugs were studied in the catfish. Glucose loading produced a dose-related hyperglycemia, maximum within 3 hr, while alloxan caused a biphasic rise in glucose level without induction of permanent diabetes. Streptozotocin elicited a monophasic hyperglycemic state at a lower dose and biphasic response at higher doses. Glybenclamide produced hypoglycemia in normal and sham-operated fish; the depancreatized animals were unresponsive to this treatment. In all the cases, normoglycemic values were restituted within 4 days of the treatment. The β-cells of the islets underwent varying histopathological changes with signs of regenerative activity. A depletion in heavy metal (zinc) in these cells was also evident after treatment with streptozotocin.

Key words : diabetogenic agents, antidiabetic drugs, blood glucose, pancreatic islets, catfish

INTRODUCTION

Teleosts with islets as grossly visible 'Brokmann Corpuscles' offer possibilities for studies on pure islet tissue. Streptozotocin and glybenclamide are used for characterization of β-cell function in lower vertebrates (1, 8, 9) and for studies involving comparative pharmacology and toxicology (11). The present study was carried out in the catfish (*Clarias batrachus*) to reinvestigate the effects of these drugs, which have short biological and chemical half-life.

MATERIAL AND METHODS

Adult seemingly-normal specimens of both sexes of *C. batrachus* were procured from the commercial source. Animals (body weight, 60–130 g; length, 11–20 cm) were maintained under the controlled conditions of light (12 hr light: 12 hr darkness) and temperature (22±1°C) and were fed with laboratory food (Aqua Food: Ashoka Fish Centre, New Delhi) *ad libitum*. Following drugs (or vehicles) were administered deep into the epaxial musculature after 24 hr of fasting at doses mentioned in Table I: (I) A 10% solution of glucose (BDH Glaxo Laboratories, India); (II) Alloxan (hexahydrate; Merck, F.R.G.) and (III) Streptozotocin (SZ) (Upjohn Co., Kalamazoo, Michigan, U.S.A.) dissolved in phos-

phatecitrate buffer at pH 4.0. Glybenclamide (Daonil*, Hoechst Pharmaceuticals Ltd., Bombay) was given orally in normal fish and sham-operated and partially-pancreatectomized fish after 48 hr of post-operative acclimation. The operations were performed as detailed elsewhere (3).

From each group, 3–4 animals were sacrificed by decapitation at the intervals varying between 0.5 hr and 10 days. The blood glucose values were assayed spectrophotometrically (13). The pancreatic tissue was fixed for 15–24 hr in Bouin's or Helly's fluids. For silver-sulfide, the tissues were fixed in cold glutaraldehyde under continuous gassing with hydrogen sulfide. The serially cut sections of paraffin-embedded tissue were subjected to differential stains (4).

RESULTS

Blood glucose (Table I) : Each dose of glucose led to an immediate, significant hyperglycemic response. Blood glucose of fish treated with alloxan rose to>100%

TABLE I : Blood glucose level (*mg%*, mean ± SEM) after treatment with some drugs in *Clarias batrachus*.

Group	*Dose*	*Blood glucose (hr after the treatment)*							
		1	3	5	10	15	24	72	120
Glucose loading	Control	55± 1.1	—	65 ±1.41	60±1.3	59±2.0	52±2.5	—	—
	1 *gm/kg*	298±11.0 (p<0.001)	—	126±5.8 (p<0.02)	78±5.0	91±8.6	58±5.7	—	—
	2 *gm/kg*	304±10.7 (p<0.001)	—	363±23 8 (p<0.001)	345±10.7 (p<0.001)	100±12.8	152±8.7 (p<0.01)	58±13.9	—
	5 *gm/kg*	335±5 3 (p 0.001)	—	346±7.6 (p<0 001)	272±9.9 (p<0.001)	210±7.5 (p<0.005)	173±13.9 (p<0.01)	60±4.0	—
Alloxan	200 *mg/kg*	74±1.5	—	87 ±5.8	111±4.1 (p<0.01)	117±4.2 (p<0.01)	85±4.0	43±6.3	—
	400 *mg/kg*	87±3.2	—	158±10.7 (p<0.005)	187±7.3 (p<0.005)	64±5.8	48±6.1	52±5.8	120±4.1 (p<0 02)
SZ	50 *mg/kg*	78±6.4	—	82 ±2.5	90±3.2	65±3.0	68 ±1.15	—	—
	100 *mg/kg*	96±1.8	105±2.4 (p<0.02)	116±3.6 (p<0.02)	86±2.2	60±0.8	100±2.0	—	62±0.5
	200 *mg/kg*	93±1.2	135±1.5 (p<0.005)	128±3.6 (p<0.01)	90±2.0	132±2.8 (p<0.005)	120±2.0 (p<0.02)	60±1.0	—
Glyben-clamide	Normal								
	2.5 *mg/kg*	66±2.0	—	46 ±7.0 (p<0.02)	—	36 ±1.0 (p<0.005)	32 ±1.0 (p<0.005)	54 ±2.8	—
	5 *mg/kg*	89±10.2 (p<0.02)	—	42 ±9.0 (p<0.01)	—	15 ±12.6 (p<0.001)	31 ±3.0 (p<0.005)	75 ±2.0	65±0.4
	Sham Depancr-eatized 5 *mg/kg*	78±1.5	—	58 ±1.4	—	38 ±1.2 (p<0.005)	45 ±2.0 (p<0.01)	59 ±1.0	—
		118±8.0	—	100±2.8	—	95 4.0	98 ±4.2	110±10.2	124±8,6

Each value represents the mean of 3–4 individual determinations.

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control within 1 hr with a peak occurring within 10 hr postinjectionum. Higher doses of SZ elicited a hyperglycemia of biphasic response, maximum within 5 hr of the treatment. Glybenclamide caused time-and dose-dependent hypoglycemia with the peak falling within 24 hr. The response of the drug was preceeded with an initial paradoxic hyperglycemic state. In partially-pancreatectomized fish the drug failed to produce glycovariations whilst in sham-controls, the response was like that of the controls. Except the alloxan-treated and the depancreatized fish, all other animals regained normoglycemia within day 4.

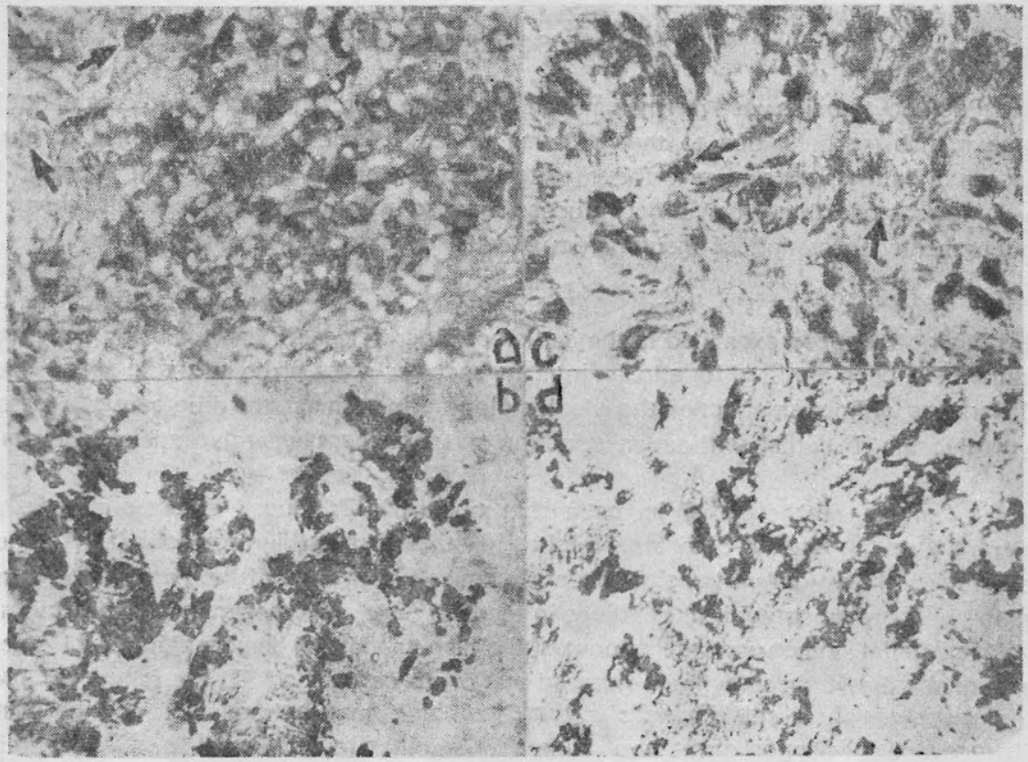

Fig. 1 : Photomicrographs of the pancreatic islets of *C. batrachus*. a—AF–light green stained preparation showing normal α-(light) and β-(dark) cells in the control fish. Magn. approx. 300 X; b—showing zinc in β-cells of control fish. Silver-sulfide. 300 X; c—alloxan-induced changes in a phosphotungstic acid-hematoxylin preparation (dose, 400 *mg/kg*). 320 X; d– showing depletion of β-cell zinc after 10 *mg/kg* dose of SZ. Silver-sulfide. 260 X.

Pancreatic islets (Figs 1 a-d) : In the untreated control groups the α and β-cells showed considerable variation in the distribution of granules within various islets of the same fish. The islets of Langerhans appeared to possess a preponderance of aldehyde-fuchsin (AF) +ve β-cells (Fig. 1 a) which contained a high content of zinc (Fig. 1b).

With the exocrine parenchyma remaining unaffected, the endocrine tissue revealed necrobiotic changes after treatment with various drugs. Following glucose and alloxan, the α and β-cells underwent hypertrophy, degranulation and vacuolation (Fig. 1c). The islets of SZ-treated fish appeared smaller with their β-cells represented by accumulations of amorphous 'debris'. Further, infiltration of a few central cells from exocrine pancreas was shown by a few islets. A conspicuous feature of such islets was the depletion in zinc granulation (Fig. 1 d). The cellular changes after glybenclamide in normal and sham-operated animals were similar to those observed after glucose loading except that vacuolation of β-cells was not seen.

DISCUSSION

Islet cytotoxins used in mammalian diabetes research have provided data of doubtful value in fish, partially due to their extreme systemic toxicity. The response of glucose loading in catfish was similar to that reported for the Nothern pike (9). It appeared from the present study that when blood glucose reached the hyperglycemic levels, there was an augmented secretion of insulin from β-cells, causing their degranulation.

In mammals (6, 15) alloxan and SZ have a brief chemical and biological half-life. This has not yet been examined in the catfish or other teleosts. Irregular and undeducible results have been reported for alloxan in many species: in the pike and the goldfish (9, 12) alloxan was without an affect on the islet histology. In contrast, the drug caused a 'passager hyperglycemia' in catfish without an induction of a permanent hyperglycemia as in mammals.

Our data in the catfish indicates that model of SZ-induced diabetes may be more relevant than the alloxan-diabetes model as in other air-breathing teleosts (8, 10) though *Esox lucius* shows only a transient hyperglycemia (9). Results of the present experiments are in good agreement with the findings in toadfish (7) and in mammals (14) in that there is close parallelism between the amount of the histochemically demonstrable β-cell zinc and functional state of these cells in diseased state. In contrast to SZ, the failure of alloxan to produce an observable changes in the β-cell zinc may be explained on the basis of different modes of action of the two drugs (15) and inability of alloxan to stimulate release of insulin from degenerating β-cells in diabetes (5).

The sensitivity to hypoglycemic action of glybenclamide differ in mammals (1). in poiklotherms, the drug exhibits β-cell cytotropic property (2) which is absent in fish (9). In the present study, the response of catfish to glybenclamide appeared to be akin to mammals though efficacy differed. Similar to what has been reported in pancreatectomized, alloxan or SZ-diabetic mammals (1, 11), the drug failed to evoke a change in blood glucose of depancreatised fish, suggesting that the drug causes an endogenous release of insulin: this was substantiated from the observed effect on islet β-cells.

The present work suggests that catfish pancreatic tissue responds to certain diabetogenic agents (like SZ) and antidiabetic agents (like glybenclamide) in much the same way as the mammalian pancreatic tissue. It will be worthwhile to examine if a catfish-diabetes model could be evolved as a primary screen for potential antidiabetic drugs.

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