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Tolbutamide-Induced Changes of the DNA, Protein and Insulin Content and the Secretory Activity of Isolated Rat Pancreatic Islets

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Summary. Following prolonged administration of tolbutamide the DNA- and protein content per islet was enhanced but the IRI content per islet was diminished. Glucose-induced (2.0, 8.0 or 16.6 mM) and leucine-induced (12.5 or 25.0 mM) IRI release from isolated islets, as well as $^{14}CO_2$-production from U-^{14}C glucose, were decreased. Theophylline (5.0 mM) restored the glucose sensitivity of the islets towards normal. The results indicate that tolbutamide-induced islet cell hyperplasia does not entail islet hyperfunction, as previously thought. Decreased IRI release may partially be explained by a tolbutamide-induced alteration of the adenylate cyclase/phosphodiesterase system of the B-cell.

Key words: Islet hyperplasia, islet DNA, islet protein, insulin release, tolbutamide treatment, theophylline.

Sulfonylurea compounds are thought to decrease blood glucose via increased insulin (IRI) release, but their detailed mode of action is still a matter of debate, particularly as far as the long-term effects of sulfonylureas are concerned (for review see ref. 1). Most studies have failed to show a correlation between decrease in blood glucose and increase in IRI levels after prolonged administration of sulfonylureas to patients [2–7]. Moreover, histomorphological observations on islet tissue after chronic administration of sulfonylureas have shown increased islet growth, which has usually been interpreted as indicative of islet *hyper*function [8 – 14], while biochemical studies indicate a diminished glucose tolerance and a decrease in IRI release and/or IRI content of the islets suggesting islet *hypo*function [15 – 19].

We reinvestigated the influence on rat pancreatic islets of prolonged administration of tolbutamide, in a dosage known to induce maximal degranulation of the B-cells within 3 to 5 days, by studying concomitantly islet composition (content of DNA, protein and IRI), metabolic activity (CO_2-production) and secretory behaviour (IRI release induced by glucose, leucine and theophylline).

Decreased IRI release was found to occur in the presence of islet hyperplasia, thereby indicating that tolbutamide-induced increase in islet size does not entail islet hyperfunction, as previously thought. One of the possible reasons for the decreased secretory activity may be an alteration in the adenylate cyclase/phosphodiesterase system of the B-cell.

Materials and Methods

Ficoll (lot 4556) was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden; 3,5-diaminobenzoic acid dihydrochloride (lot A2A) from Eastman Kodak Co., Rochester, New York, USA; salmon sperm DNA (lot 15 776) from Boehringer Mannheim GmbH, Mannheim, FRG; Hyamine-hydroxide from Koch-Light Lab. Ltd, Colnbrock, England; Dimilume from Packard Instrument Co., Warrenville, Illinois, USA; Altromin from Spezialfutterwerke, Lage, FRG; U-^{14}C glucose (0.25 mC/13.4 mg, lot 299 – 022) from New England Nuclear Inc., Boston, Massachusetts, USA. Further reagents used are listed in a previous report [20].

Male Wistar rats fed Altromin and water ad libitum received Na-tolbutamide (Rastinon^{R}) 500 mg/kg twice daily for 7–9 days or for 29–54 days by an oesophageal tube. The mean initial body weight of long-term treated animals both in the tolbutamide group (n = 28) and the controls (n = 28) was 223 g, while the final body weight was 275 vs 278 g, respectively. The mean initial body weight of short-term treated animals, both in the tolbutamide group (n = 10) and the controls (n = 10), was 218 g, while the final body weight was 238 vs 232 g respectively.

Islets, isolated from rat pancreas by collagenase digestion [21] and purified by a discontinuous Ficoll gradient [22], were incubated as previously described [20] with concentrations of glucose, leucine and the-

ophylline indicated in the tables. The average number of islets collected per pancreas of tolbutamide-treated and control rats was nearly identical (305 vs. 290). IRI from the medium was determined by radioimmunoassay with rat insulin as a standard [23]. Production of $^{14}CO_2$ from U-^{14}C glucose (specific activity in the medium 0.12 mC/mmol glucose) was measured by incubating groups of 25 islets for 3 h in flasks holding a glass well and closed with a rubber stopper. Experiments were preceded by a 5 min equilibration period with an O_2-CO_2 mixture (95%:5%) and terminated by injecting 0.2 ml of 10 N H_2SO_4 into the medium and 0.5 ml of Hyamine-hydroxide into the glass well. After a collection period of 90 min Hyamine was transferred to scintillation vials containing 10.0 ml Dimilume. Radioactivity was measured in a Beckman Tricarb liquid scintillation Spectrometer (model 3380) equipped with a computer unit (model 544) for quench correction by external standardisation. Extraction and measurement of total IRI, alkali soluble protein and DNA from islets was as described by Green and Taylor [24].

Results

The protein content of islets of rats treated for 7–9 days with tolbutamide was almost identical to that of control islets, whereas the extractable IRI content was significantly lower. By contrast, islets from rats treated for at least 29 days with tolbutamide and sacrificed 24 or 2 h after the last dose had a higher protein and DNA content than the appropriate controls, while their IRI content was lower. The protein/DNA ratio was found to be nearly identical in each of the four groups (Table 1).

Islets from these long-term treated rats produced $^{14}CO_2$ from U-^{14}C-glucose at a lower rate than control islets, both at low (5.0 mM) and high (16.6 mM) glucose concentration. In the presence of 5.0 mM glucose islets from tolbutamide-fed rats generated 172 ± 12 (n = 16) compared with 267 ± 22 (n = 19) dpm/µg DNA in controls ($P < 0.01$). In the presence of 16.6 mM glucose the respective values were 741 ± 55 (n = 18) vs. 939 ± 70 (n = 18) dpm/µg DNA ($P < 0.025$). The n-values indicate the number of individual observations obtained from 10 pancreata per group. The animals were sacrificed 24 h after the last tolbutamide dose.

Glucose-induced (2.0, 8.0 or 16.6 mM) IRI release was lower from islets of tolbutamide-treated rats than from the appropriate controls. The respective differences were more pronounced 24 h than 2 h after the last dose. In addition, leucine-induced (12.5 or 25.0 mM) IRI release was lower from islets of tolbutamide-fed rats compared with controls (Table 2).

The addition of theophylline (5.0 mM) to the glucose stimulus (16.6 mM) enhanced and nearly normalized the diminished IRI release from islets isolated 24 or 2 h after the last tolbutamide dose. By contrast, theophylline had only slight additional effects on glucose-induced (16.6 mM) IRI release from control islets (Table 3). The mean increment of IRI release 2 h after tolbutamide was 11.6 ng/10 islets/45 min (28%) vs 5.2 ng/10 islets/45 min (11%) in control islets. 24 h after tolbutamide the mean increment was 25.8 ng/10 islets/45 min (45%) vs 7.0 ng/10 islets/45 min (11%) respectively.

Discussion

Our data show an increase in the amount of extractable DNA and protein from islets following prolonged administration of tolbutamide, thereby indicating an increase in islet size. This increase in islet size seems to be due to an increase in cell number rather than in individual cell size, i. e. islet cell hyperplasia, because the protein/DNA ratio remained almost unchanged (Table 1). Our findings are compatible with the histomorphological observation of increased islet growth following prolonged administration of

Table 1. *Effect of long-term administration of tolbutamide on the content of insulin, DNA and protein from isolated rat islets. Mean values ± SEM are shown with the number of individual observations obtained from 10–15 pancreata in parentheses. An asterisk indicates a significant difference from the respective control value (p < 0.05 or less). Values for "P" were calculated by the "t" test based on nonpaired comparisons*

	Feeding period (days)	Last tolbutamide dose prior to sacrifice (h)	DNA (ng/islet)	Protein (ng/islet)	Protein/DNA (ratio)	Insulin (ng/islet)
Tolbutamide	7–9	24	–	529 ± 31 [20]	–	60 ± 4 [14]*
Control	7–9	24	–	548 ± 14 [20]	–	77 ± 7 [12]
Tolbutamide	29–54	2	58.0 ± 1.2 [40]*	774 ± 17 [23]*	13.5	56 ± 7 [11]*
Control	29–54	2	40.8 ± 1.0 [33]	570 ± 16 [25]	13.9	74 ± 5 [11]
Tolbutamide	29–54	24	56.0 ± 1.2 [40]*	741 ± 43 [14]*	13.2	54 ± 6 [10]*
Control	29–54	24	41.5 ± 1.1 [20]	550 ± 23 [14]	13.3	74 ± 8 [10]

Table 2. *Effect of long-term administration of tolbutamide (29–54 days) on glucose- and leucine-induced IRI release from isolated rat islets. Mean values ± SEM are shown with the number of individual observations obtained from 10–15 pancreata in parentheses. An asterisk indicates a significant difference from the respective control value (p < 0.025 or less). Studies on rats sacrificed 24 h after the last tolbutamide dose were done about 8 weeks later than the experiments with rats sacrificed 2 h after the last dose. Values for "P" were calculated by the "t" test based on nonpaired comparisons*

Last tolbutamide dose	Glucose	Leucine	IRI release (ng/10 islets/45 min)		Difference
prior to sacrifice (h)	(mM)	(mM)	Tolbutamide	Control	(%)
2	2.0	–	9.3 ± 0.5 [34]*	12.9 ± 0.7 [31]	28
2	8.0	–	14.5 ± 1.0 [17]*	23.2 ± 2.2 [14]	38
2	16.6	–	29.0 ± 2.4 [17]*	39.6 ± 2.6 [17]	26
24	2.0	–	5.4 ± 0.7 [15]*	10.8 ± 1.1 [15]	50
24	8.0	–	10.0 ± 2.4 [11]*	21.7 ± 1.2 [12]	54
24	16.6	–	20.0 ± 1.4 [12]*	53.0 ± 2.4 [12]	63
2	–	12.5	14.8 ± 1.3 [15]*	17.7 ± 0.7 [15]	17
2	–	25.0	22.7 ± 2.0 [14]*	30.9 ± 3.8 [12]	27

Table 3. *Effect of theophylline on glucose-induced IRI release from isolated islets of rats long-term fed with tolbutamide. Mean values ± SEM are shown with the number of individual observations obtained from 10–12 pancreata in parentheses. An asterisk indicates a significant difference from the respective control value (p < 0.01). Values for "P" were calculated by the "t" test based on nonpaired comparisons*

Last tolbutamide dose	Glucose	Theophylline	IRI release (ng/10 islets/45 min)		Difference
prior to sacrifice (h)	(mM)	(mM)	Tolbutamide	Control	(%)
2	16.6	–	29.5 ± 2.1 [20]*	42.4 ± 2.0 [20]*	30
2	16.6	5.0	41.1 ± 1.7 [40]	47.6 ± 2.7 [40]	14
24	16.6	–	22.0 ± 1.3 [16]*	54.0 ± 2.2 [16]*	59
24	16.6	5.0	47.8 ± 1.6 [40]	61.0 ± 1.8 [40]	22

tolbutamide to rats [8–11, 13], rabbits [12] and dogs [14]. This increased islet growth has usually been interpreted as indicative of islet hyperfunction, i.e. increased sensitivity of the mechanism responding to glucose, thereby supporting the concept of a direct beta-cytotrophic effect of sulfonylurea derivatives as the cause for the lasting blood glucose lowering action. In support of the assumption that increased islet growth may be indicative of islet hyperfunction, increased IRI release was reported from islets with hyperplasia due to pregnancy [24]. However, our data indicate that islets with hyperplasia as the result of chronic administration of tolbutamide release less IRI after stimulation with glucose or leucine than the appropriate controls (Table 2 and 3). This confirms and extends previous reports [16]. Tolbutamide-induced increase in islet size is thus not evidence for islet hyperfunction, as previously thought.

The reason for the tolbutamide-induced hyperplasia remains unclear, unless it is a compensatory mechanism due to the decrease of IRI content (Table 1). A low IRI content in islets could be explained by either decreased IRI synthesis and/or by continuously increased IRI release. Diminished proinsulin/insulin synthesis has been found both after administration of tolbutamide *in vitro* [25–27] and after chronic administration of various sulfonylureas *in vivo* [16]. Whether this is reflecting a specific inhibitory effect of tolbutamide on IRI synthesis, or rather a depression of overall islets metabolism, is not known. Impaired glucose metabolism in isolated islets could be expected to interfere with IRI synthesis. Diminished glucose oxidation in islets from longterm tolbutamide-fed rats has been reported and is confirmed here. Further data on the effect of chronic administration of sulfonylureas on islet cell metabolism are lacking. *In vitro* administration of sulfonylurea compounds seems to lower the ATP and the glucose-6-phosphate content of isolated islets [28, 29] even though the islet oxygen consumption and lactate production is increased [30, 31]. Thus, despite some evidence to the contrary [32, 33], sulfonylureas

may uncouple oxidative phosphorylation in islets, thereby inhibiting energy producing reactions required for IRI synthesis. The low IRI content in islets from long-term tolbutamide-fed rats may therefore be explained by diminished IRI synthesis. Continuously increased IRI release does not seem to be a contributing factor.

IRI release is diminished in response to glucose and leucine (Table 2). This diminished secretory response to a glucose or leucine stimulus could be the consequence of the low synthetic activity and the decreased availability of IRI. If this is the only possible explanation, major changes in the secretory pattern of the islets should not be observed. However, there was an alteration in the theophylline-sensitivity of these islets. Theophylline enhanced the effect of a glucose stimulus (16.6 mM) on IRI release significantly more in islets from tolbutamide-fed rats than from controls (Table 3).

The combined effects of theophylline and glucose on IRI release have repeatedly been investigated and have led to conflicting results. Theophylline was reported to stimulate IRI release in the presence of a low glucose concentration (1 mg/ml) [34], an effect not seen by others [35]. Furthermore, while theophylline was found to stimulate IRI release much more in the presence of high than of low glucose concentrations [35], there are also observations to the contrary [36]. In the presence of a high glucose concentration (16.6 mM) we found a small effect of theophylline on IRI release. However, these various observations reflect differences mainly in the quantity but not in the quality of the observed effects.

Theophylline is thought to modulate the secretory process of the B-cell via the adenylate cyclase/ phosphodiesterase system [37–42]. The lowered sensitivity of the B-cell insulin releasing mechanism induced by prolonged treatment with tolbutamide may, therefore, be partially effected by an interference with the enzymatic system generating and inactivating cyclic adenosine monophosphate. However, studies on the effect of sulfonylureas on islet tissue should consider that normal and diabetic islet tissue might act differently towards these agents, as recently suggested [43].

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