SHORT COMMUNICATIONS

Experimental Studies on the Glycogen Content and the Incorporation of Radioglucose into the Glycogen of the Diaphragm of Rats after Initial and Short Term Administration of Buformin

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Summary. After a short term oral administration of 25 mg buformin per animal (100–125 mg/kg/day) and final intraperitoneal injection of glucose we found a significantly increased content of glycogen in the diaphragm of normal male and female rats together with a higher incorporation of glucose. The latter effect was not observed if the last dose of buformin was given 24 h before the investigation. No effect on glycogen content or glucose incorporation into the glycogen was measurable after

daily administration of 2.5 mg buformin per animal for 14 days or a single dose of 25 mg buformin per animal.

The present investigation confirms the clinical experience that the effect of biguanides is seen only after some days of treatment.

Key words: Biguanide, radioglucose incorporation, glycogen, rat diaphragm.

Introduction

Very little is known of the mechanism of action of the blood glucose lowering biguanides. From numerous experimental data it may be concluded that more than one process is involved (Creutzfeldt *et al.*, 1970). There are three sites postulated: —

1. Potentiation of insulin-induced glucose uptake in peripheral tissue.
2. Inhibition of increased gluconeogenesis in the liver.
3. Inhibition of intestinal absorption of glucose and other substances actively transported.

In vitro, biguanides enhance glucose uptake by isolated rat diaphragm (Williams *et al.*, 1957; Steiner and Williams, 1959; Ungar, 1959; Rafaelsen and Lundbaek, 1959; Clarke and Forbath, 1959; Daweke and Bach, 1963). Maximum effects were obtained at biguanide concentrations of 10^{-3} M.

In contrast to increased glucose uptake, biguanides do not stimulate glycogen formation from glucose but increase glycolytic breakdown of glucose to lactate (Williams *et al.*, 1957; Steiner and Williams, 1959; Rafaelsen, 1959; Rafaelsen and Lundbaek, 1959; Clarke and Forbath, 1959). Concomitantly a decreased oxygen uptake has been observed.

In vivo studies in which 0.5–120 mg/kg biguanides were administered as a single dose either orally (Liebermeister and Daweke, 1969) or intraperitoneally, together with $[U-^{14}C]$ glucose (Ditschuneit and Ditschuneit, 1965), showed no effect on the incorporation of glucose into the glycogen of the rat diaphragm. On the other hand Creutzfeldt *et al.* (1961)

found that biguanides potentiated the effect of insulin in eviscerated rats. Losert *et al.* (1970) showed that the incorporation of $[U-^{14}C]$ glucose into the glycogen of rat diaphragm was significantly higher after oral administration of 2.35 mg buformin/kg for 3 to 14 days as compared with a placebo treated group. At the same time they observed an increased glucose oxidation measured as $^{14}CO_2$ in the expired air. In other experimental studies a stimulating effect of biguanides on glucose oxidation was also described by Dobson and Allen (1967), Beckmann (1968) and Searle and Cavalieri (1968).

Butterfield *et al.* (1958) demonstrated in vivo that 7–10 days oral treatment with phenformin increased peripheral glucose uptake in the forearm muscle of obese diabetics by reducing the glucose threshold.

From clinical experience it is known that the effect of biguanides is seen only after several days treatment (Weinges *et al.*, 1970). We (Strohfeldt *et al.*, 1970) presented preliminary results that after short term treatment with 25 mg buformin per day an increased incorporation of glucose into the glycogen of rat diaphragm was observed. The purpose of the present investigation was to study the glycogen content and the incorporation of glucose into the glycogen of the diaphragm of rats after a single and a short term administration of buformin.

Methods

A total of 185 Sprague-Dawley rats of both sexes (predominantly males) with a weight of 150–180 g were used. They were kept on a commercial diet ad

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libitum. One group of animals were given a single dose of 25 mg buformin per stomach tube and killed 2 h later (one time trial). The other groups received 2.5 mg or 25 mg buformin daily for 12 to 14 days (short term trial). The last buformin dose was given either 2 h or 24 h before they were killed. All experiments were carried out with a control group, which received 0.9% saline by stomach tube.

25 mg glucose together with 2 μ Ci [$U^{14}C$] glucose (sp. activity 3 mCi/mM) was administered intraperitoneally in order to stimulate endogenous insulin secretion two hours before the investigation. One study was carried out with an injection of exogenous insulin (100 μ U) instead of glucose. The time of day of drug administration and investigation was identical in all trials.

The glycogen was determined by a modified method of Walaas and Walaas (1950). The animals were killed by decapitation, the diaphragms rapidly removed, washed in ice cooled 0.9% saline, blotted on filter paper and weighed. These were then transferred to 1 ml of 30% KOH and digested for 10 minutes at 100°C and 1.2 ml alcohol was added. After standing in ice for 15 min, the glycogen was removed by centrifugation, the supernatant fluid decanted and after adding 1 ml water, the precipitation with 1.2 ml alcohol was repeated. The last traces of alcohol were expelled by drying in an incubator at 50°C for 15 min and the precipitate was dissolved in 1 ml of 5N HCl. After hydrolysis for 30 min at 100°C the contents were neutralized with 2N NaOH, water was added to a final volume of 10 ml and aliquots were taken for glucose determination. This was analysed by the glucose oxidase-method. The counting was performed with the tricarbscintillation counter after adding 9 ml of Bray's scintillation fluid to a 1 ml aliquot. The glycogen content was expressed as mg glycogen per 1 g diaphragm wet weight (ww) and the activity of radioglucose as dpm (10^3) per 1 g diaphragm ww.

The results of the treated groups were compared to those of the control groups using the Wilcoxon-test.

Results

In the group which received a single dose of 25 mg buformin per animal no effect on the glycogen content of the diaphragm and the incorporation of glucose into the glycogen of the diaphragm was observed (Table 1).

There was also no influence upon the two measured parameters in the animals pretreated for 14 days with the low dose of 2.5 mg buformin/animal (Table 2). The results given in Table 3 show an increased glycogen content of the diaphragm and a higher rate of incorporation of glucose in the glycogen after oral administration of 25 mg buformin/day/animal for 14 days, provided the animals received the last dose of buformin 2 h before the investigation. The same results

were obtained from studies with rats also being pretreated with 25 mg buformin per day per animal for 14 days. Instead of a stimulation of endogenous insulin

Table 1. *One time trial: Glycogen in mg/g diaphragm ww and activity of the incorporated glucose in dpm (10^3)/g diaphragm ww after a single oral treatment with 25 mg buformin and intraperitoneal injection of 25 mg glucose together with 2 μ Ci [$U^{14}C$]-glucose 2 h before the investigation*

$\bar{X} \pm S_{\bar{x}}$	mg glycogen/ g diaphragm ww	dpm (10^3)/ g diaphragm ww	n
Control	2.99 ± 0.18	2.93 ± 0.50	10
Buformin (25 mg)	3.47 ± 0.26	2.89 ± 0.96	20
	$p > 0.01$	$p > 0.01$	

Table 2. *Short term trial: Glycogen in mg/g diaphragm ww and activity of the incorporated radioglucose in dpm (10^3)/g diaphragm ww after oral treatment with 2.5 mg buformin/animal for 14 days. Last dose 2 h before investigation. At the same time intraperitoneal injection of 25 mg glucose together with 2 μ Ci [$U^{14}C$]-glucose*

$\bar{X} \pm S_{\bar{x}}$	mg glycogen/ g diaphragm ww	dpm (10^3)/ g diaphragm ww	n
Control	3.44 ± 0.15	6.02 ± 0.71	10
Buformin (2.5 mg)	3.04 ± 0.15	4.29 ± 0.64	14
	$p > 0.01$	$p > 0.01$	

Table 3. *Short term trial: Glycogen in mg/g diaphragm ww and activity of the incorporated radioglucose in dpm (10^3)/g diaphragm ww after oral treatment with 25 mg buformin/animal for 14 days. Last dose 2 h before investigation. At the same time intraperitoneal injection of 25 mg glucose together with 2 μ Ci [$U^{14}C$] glucose*

$\bar{X} \pm S_{\bar{x}}$	mg glycogen/ g diaphragm ww	dpm (10^3)/ g diaphragm ww	n
Control	2.18 ± 0.14	2.95 ± 0.56	15
Buformin (25 mg)	4.79 ± 0.48	8.33 ± 2.03	22
	$p < 0.01$	$p < 0.05$	

Table 4. *Short term trial: Glycogen in mg/g diaphragm ww and activity of the incorporated radioglucose in ipm (10^3)/g diaphragm ww after oral treatment with 25 mg buformin/animal for 14 days. Last dose 2 h before investigation. At the same time intraperitoneal injection of 100 μ U insulin together with 2 μ Ci [$U^{14}C$]-glucose*

$\bar{X} \pm S_{\bar{x}}$	mg glycogen/ g diaphragm ww	ipm (10^3)/ g diaphragm ww	n
Control	2.78 ± 0.34	0.84 ± 0.31	6
Buformin (25 mg)	7.26 ± 1.16	3.68 ± 1.02	8
	$p < 0.01$	$p < 0.05$	

secretion with 25 mg glucose, these animals received an injection of 100 μ U insulin intraperitoneally together with 2 μ Ci [$U^{14}C$] glucose 2 h before investigation (Table 4).

The results given in Table 5 show the glycogen content and the incorporation of glucose into the diaphragmatic glycogen in those animals which received 25 mg buformin for 12 days and the last dose 24 h before the investigation. The results show that the total glycogen content was considerably higher in the diaphragm from the treated animals than in that of the untreated animals. There was, however, no

c) if a single dose of buformin is administered neither parameter is affected.

It is concluded that to achieve effects upon muscle glycogen metabolism chronic administration of this drug is essential and the final acute dose must not be given more than a few hours before the investigation.

Table 5. Short term trial: Glycogen in mg/g diaphragm ww and activity of the incorporated radioglucose in dpm (10^3)/g diaphragm ww after oral treatment with 25 mg buformin/animal for 12 days. Last dose 24 h before investigation. 2 h before investigation intraperitoneal injection of 25 mg glucose together with 2 μ Ci [$U\text{-}^{14}C$] glucose

$\bar{X} \pm S_{\bar{x}}$	mg glycogen/ g diaphragm ww	dpm (10^3)/ g diaphragm ww	n
Control	3.85 ± 0.28	4.13 ± 0.95	13
Buformin (25 mg)	6.70 ± 1.02	4.91 ± 1.31	14
	$p < 0.01$	$p > 0.05$	

difference in the incorporation of glucose into the glycogen of the diaphragms. In the three groups pretreated for 12–14 days with 25 mg buformin/day/animal about 50% of the animals died during the time of treatment. It is probable that hypoglycemia was the reason for this and some rats found dying were noted as having blood glucose level of 4–16 mg%.

Discussion

After the oral administration of 25 mg buformin per animal for 12–14 days (100–125 mg/kg/day) and a final intraperitoneal injection of glucose it was found that the glycogen content of the diaphragm was significantly increased and there was a higher incorporation of glucose when compared with the control groups. This latter effect was not observed if the last dose of buformin was given 24 h, instead of 2 h before the investigation. 50% of the treated animals died during the short term administration of 25 mg buformin per animal.

Neither the "one time trial" with 25 mg buformin per animal nor the short term trial with 2.5 mg buformin per animal influenced the two parameters.

There was no difference shown between male and female rats.

The major conclusion of this paper is therefore based on these three features:

- the dose of buformin when chronically administered cannot be terminated more than two hours before the investigation if both parameters are to be affected.
- if buformin dosage is terminated 24 h before investigation only the glycogen content is affected.

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