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Effects of Buformin on the Metabolism of the Isolated Haemoglobin-Free Perfused Hindlimb of Normal Rats*

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Summary. Using the isolated, haemoglobin-free, perfused resting hindlimb of normal rats buformin neither had a direct insulin-like effect on glucose uptake by muscle tissue nor potentiated the effect of insulin on glucose uptake after oral pretreatment for one or several days with low and high doses (30 mg–350 mg/kg). Effects on glycogenolysis could not be detected. Glycerol release was inhibited after several days of pretreatment with low and high doses of buformin. The utilization of added oleate was also partly inhibited. The level of energy rich phosphates in the muscle tissue and oxygen consumption were not affected under any of the conditions used in these experiments.

Key words: Buformin, muscle metabolism, isolated perfused hindlimb of the rat.

The mode of action of the biguanides is still under discussion. In recent studies from this laboratory concerning the effect of buformin on muscle metabolism we observed an increased glycogen content and a high rate of radioglucose incorporation into the glycogen of the diaphragm of normal rats *in vivo* after a long term oral administration of buformin – 150–175 mg/kg – [12]. Similar results were reported with the perfused rat heart [15] and in *in vivo* experiments [11]. In order to investigate a direct action of buformin on glucose utilization in skeletal muscle, the isolated perfused hindlimb of the rat was used as a suitable model for metabolic studies [13, 14]. In this study we report the effect of low and high doses of buformin, administered orally for one or several days, on the metabolism of the perfused resting hindlimb of normal rats.

Materials and Methods

Male, fed Sprague-Dawley rats weighing 160–240 g were used. Buformin (kindly supplied by the Chemie Grünenthal GmbH, Stolberg, Rhld., Germany) was dissolved in physiological saline (solutions of 5 and 10 per cent respectively) and administered daily via a stomach tube. The doses ranged from 30 to 350 mg/kg. The control animals received physiological saline. The perfusion lasted for 1 h and started 2.5 h after the last drug administration. The perfusion technique, apparatus, synthetic medium and analytical methods used have been reported in detail elsewhere [13, 14].

In addition, the standard medium contained oleate (1 mM, ac.ol. E. Merck, Darmstadt, Germany). Oleic acid was added as an emulsion [9, 10]. Free fatty acids (FFA) were determined by the method of Dole and Meinertz [8]. In the perfusion with insulin (crystalline bovine insulin, Hoechst AG, Frankfurt/M., Germany), the hormone was added at the beginning of the perfusion period in a concentration of 100 μU/ml medium. The Wilcoxon test was used for statistical analysis.

Results

The basal glucose uptake was not increased by buformin (Table 1). When the glucose uptake was stimulated by submaximal concentrations of insulin, the glucose uptake significantly decreased after prior oral administration of $3-5 \times 175$ mg buformin/kg (Table 2). The lactate production did not increase significantly. Glycogenolysis could not be detected with or without insulin in the medium.

The glycerol release was lowered after several days of pretreatment with low and high doses of buformin, regardless of the addition of insulin (Tables 1 and 2). The decrease of the concentration of oleate in the medium was less with low and high doses of buformin, but inconsistently (Tables 1 and 2). The content of the energy-rich phosphates in the muscle tissue after perfusion was unaffected under all conditions, as was the oxygen consumption (control values:

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ATP 7.8 ± 0.3 μmol/g, creatine phosphate: 20.0 ± 1.5 μmol/g, oxygen consumption: 27.0 ± 0.8 μmol/g × h).

Discussion

In the present study, buformin did not increase basal or insulin stimulated glucose uptake of the perfused hindlimb of normal rats after oral pretreatment for one or several days. In contrast, after the administration of 3–5 × 175 mg/kg buformin insulin stimulated glucose uptake decreased significantly. This is in contrast with the findings of most of the authors working with the isolated diaphragm (for a review see 1). Similar results were observed in the perfused rat heart [15]. In this study, phenformin was added to the medium at concentrations ranging from 0 to 250 μg/ml. A slightly increased glucose uptake occurred only with 40 and 50 μg/ml. However, perfused hearts from rats starved for 48 hours showed a decreased glucose uptake with 50 μg/ml phenformin in the medium. Using the forearm method a decreased glucose utilization was also observed in normal human subjects after oral pretreatment with phenformin for 3 days [7]. With the same model biguanides increased

Table 1. *Effect of buformin on the metabolism of the perfused hindlimb of normal rats after oral pretreatment for one or several days. The standard medium contained 10 mM glucose, 1 mM lactate, 0.15 mM pyruvate, and 1 mM oleate. The glycogen content of the muscle tissue was determined at the end of the perfusion period. Results are expressed as* $\bar{X} \pm S_{\bar{X}}$, *the numbers of observations in parentheses*

pretreatment	Glucose uptake μmol/g×h	Lactate production μmol/g×h	Glycogen content μmol/g	Glycerol release μmol/g×h	FFA uptake μmol/g×h
control	8.4 ± 0.3 (12)	8.3 ± 0.7 (12)	36.3 ± 1.8 (11)	0.31 ± 0.01 (12)	0.64 ± 0.05 (12)
1 × 100 mg/kg buformin	7.1 ± 0.6 (6)	7.5 ± 1.3 (6)	36.8 ± 1.6 (6)	0.31 ± 0.05 (6)	0.41 ± 0.08[a] (6)
1 × 250 mg/kg buformin	9.6 ± 0.9 (4)	9.6 × 0.5 (4)	46.7 ± 4.5 (4)	0.34 ± 0.02 (4)	0.71 ± 0.06 (4)
1 × 350 mg/kg buformin	7.6 ± 0.6 (5)	9.1 ± 0.8 (5)	53.9 ± 4.6[a] (5)	0.25 ± 0.006 (5)	0.55 ± 0.05 (5)
7 × 100 mg/kg buformin	6.9 ± 0.5 (5)	8.3 ± 0.5 (5)	40.6 ± 3.5 (5)	0.20 ± 0.02[a] (5)	0.26 ± 0.06[a] (5)
3–5 × 175 mg/kg buformin	7.5 ± 0.2 (4)	10.5 ± 0.7 (4)	46.3 ± 9.7 (4)	0.21 ± 0.03 (3)	0.46 ± 0.02[a] (4)

Table 2. *Effect of buformin on the metabolism of the perfused hindlimb of normal rats after oral pretreatment for one or several days in the presence of insulin (100 μU/ml). For further details see legend Table 1*

pretreatment	Glucose uptake μmol/g×h	Lactate production μmol/g×h	Glycogen content μmol/g	Glycerol release μmol/g×h	FFA uptake μmol/g×h
100 μU insulin ml medium	13.7 ± 0.6 (5)	11.5 ± 0.7 (5)	39.8 ± 3.7 (5)	0.36 ± 0.02 (5)	0.90 ± 0.02 (5)
7 × 30 mg/kg buformin + 100 μU insulin/ml	11.4 ± 0.8 (5)	10.2 ± 0.7 (5)	46.9 ± 2.1 (3)	0.27 ± 0.006[a] (5)	0.76 ± 0.08 (5)
7 × 100 mg/kg buformin + 100 μU insulin/ml	12.4 ± 0.6 (7)	9.1 ± 0.6[a] (7)	45.6 ± 3.9 (7)	0.16 ± 0.02[a] (7)	0.88 ± 0.06 (7)
3–5 × 175 mg/kg buformin + 100 μU insulin/ml	10.8 ± 0.7[a] (7)	11.2 ±0.6 (7)	52.2 ± 4.0 (3)	0.25 ± 0.02[a] (7)	0.68 ± 0.02[a] (7)

[a] significantly different from control ($p < 0.05$)

glucose utilization and the effect of insulin in diabetic subjects [2, 3, 4].

In our experiments, the glycogen content of the hindlimb muscle at the end of the perfusion was not affected when compared to the control perfusions. It is not possible to establish glucose balances in our experiments because there was no information available on the glycogen content of the perfused muscle at the beginning of the perfusion period. Using the isolated diaphragm, a glycogen breakdown was always observed in the presence of biguanides (for a review see 1). With *in vivo* experiments (11, for a review see 1) or with the perfused rat heart [15] the glycogen changes were small or there was an elevation of glycogen content under the influence of biguanides. We therefore assume that the increased glucose uptake and glycogen breakdown found with the isolated diaphragm under the influence of biguanides results rather from the unfavourable model than from the biguanides. Since no similar investigations have been published concerning the effect of biguanides on muscle metabolism of the isolated perfused resting hindlimb of normal rats, it is difficult to compare our data with those coming from other *in vitro* and *in vivo* experimental models.

The antilipolytic action we observed is known from *in vitro* studies with adipose tissue (for a review see 1). The utilization of the added oleate was partly inhibited after pretreatment with buformin. It cannot be decided from our experiments whether this effect is caused by a decreased activation of the fatty acid or by an inhibition of its oxidation. With isolated guinea pig heart mitochondria a decreased oxidation of acyl-CoA derivatives of long chain fatty acids and of palmitylcarnitine was observed under the influence of phenformin [5, 6]. Similar results were obtained in *in vivo* experiments after several days of pretreatment of normal rats with phenformin using an intravenous injection of 14C-1-palmitate [11].

The level of energy-rich phosphates was not affected under all conditions. In the perfused rat heart no effect on the intracellular ATP concentration was found with 50 μg/ml phenformin after 30 min of perfusion; after 60 min perfusion, however, a significant decrease was observed [15]. Buformin had no effect on the general oxygen consumption. It is not possible from our data to calculate oxidation rates for the substrates employed.

We conclude from our observations that buformin after oral pretreatment for one or several days with low and high doses has neither a direct insulin-like effect on glucose uptake by the muscle tissue of normal rats nor potentiates the effect of insulin on glucose uptake.

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