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Effects of the diuretic furosemide on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat

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Summary. The diuretic furosemide (0.5 mmol/l) impaired glucose uptake in the soleus muscle of the rat by decreasing the sensitivity of glycolysis to insulin. In addition, at higher concentrations (1.0 mmol/l) the drug inhibited the basal rate of glycolysis. It did not, however, inhibit the rate of glycogen synthesis except at a concentration of 6.0 mmol/l. Since furosemide has some structural similarities to adenosine, the above effects on insulin sensitivity may be due to its ability to act as an adenosine receptor agonist in muscle. These effects of furosemide in skeletal muscle may contribute to the glucose intolerance following therapy with this and similar agents in man.

Key words: Furosemide, insulin sensitivity, skeletal muscle, adenosine receptor agonists.

Diuretics are one of the most widely used drugs and they are administered chronically in a number of pathological conditions [1]. Despite this, they have been reported to impair glucose tolerance in normal rats and man [2-5] and to aggravate the control of the blood glucose level in patients with diabetes mellitus [6-10]. The mechanism(s) for this effect have not been established. These drugs decrease the rate of insulin secretion from incubated islets of Langerhans [11], so that failure to secrete sufficient insulin may explain the impaired glucose tolerance. However, they also cause hyperglycaemia in either pancreatectomised dogs or alloxan diabetic mice [12]: this suggests a peripheral effect of these drugs.

Recently, two of the most commonly administered diuretics, furosemide and hydrochlorothiazide, have been shown to decrease the rate of glucose transport across the cell membranes of rat adipocytes [13]. Since skeletal muscle is quantitatively the most important tissue for uptake of glucose in response to a glucose load [14, 15], it was considered important to investigate the effects of these drugs on the rates of glucose uptake and on the sensitivity of this process to insulin in muscle. Therefore, the effects of the diuretic furosemide on the sensitivity of glycolysis and glycogen synthesis to insulin concentrations in the isolated incubated soleus muscle of the rat have been studied.

Materials and methods

Male Wistar rats 160-180 g, purchased from Olac, Bicester, UK were used: they were kept in the Department's animal house for 14 days with access to food and water ad libitum prior to use. Rats were fasted for 12-14 h prior to each experiment and they were killed by cervical dislocation.

All chemicals, biochemicals and enzymes were obtained from sources given previously [16] except that furosemide, dimethylsulfoxide and dowex-2 (chloride form, dry mesh 200-400) were obtained from Sigma Chemical Company, London, UK. D-[U-^{14}C]-glucose was obtained from DuPont, Stevenage, Herts, UK. Soleus muscle strips were prepared as previously described [17, 18]. The isolated muscles were transferred immediately into siliconised 25 ml Erlenmeyer flasks containing Krebs-Ringer bicarbonate buffer at 37 °C, of the following compositon (in mmol/l): NaCl 104, Hepes 6.7, $NaHCO_3$ 22, KCl 4, $CaCl_2$ 1.1, KH_2PO_4 1, $MgSo_4$ 1, pyruvate 5, succinate 5, L-glutamate 5, D-glucose 5.5. Defatted bovine serum albumin [19] was added to a final concentration of 1.5% and the pH was adjusted to 7.31. The medium was gassed with O_2/CO_2 (95/5, v/v) during preparation; flasks were gassed with O_2/CO_2 continuously during the incubation. After 30 min preincubation the muscles were transferred into other flasks with identical Krebs-Ringer bicarbonate buffer without pyruvate, succinate or L-glutamate, containing D-[U-^{14}C]-glucose (0.5 μCi/ml) and various concentrations of insulin (1-10,000 mU/l). Furosemide was dissolved in dimethylsulfoxide at concentrations of 0.05 mol/l, 0.1 mol/l or 0.6 mol/l and was added into both the preincubation and incubation flasks at a final concentration of 0.5, 1.0, or 6.0 mmol/l. Dimethylsulfoxide was also added into the control flasks at the same final concentration as in the flasks containing furosemide (1%). After 60 min incubation the muscles were quickly removed, blotted and freeze-clamped in liquid N_2 and

processed for determination of the extent of [U-^{14}C]-glucose incorporation into glycogen [20]. The concentration of lactate in the incubation medium was assayed enzymatically [21]. ^{14}C-Lactate in the incubation medium was separated using dowex-2 (formate form) ion-exchange chromatography and the radioactivity measured in a liquid scintillation counter [22]. The rate of glucose phosphorylation (G) was calculated from the rate of total lactate formation (J), ^{14}C-lactate formation (λ) and ^{14}C incorporation into glycogen (B) using the formula $G=[(J+B)/J]\lambda$ [23].

Statistical analysis

Results in text, figure and tables are expressed as mean + SEM. Statistical significance was analysed by non-paired Student's t-test.

Results

Furosemide (0.5 mmol/l) had no effect on the rates of glycolysis at basal or maximal concentrations of insulin but decreased markedly those rates as compared to controls at 100 mU/l (7.90 ± 0.43 vs 10.13 ± 0.43 $\mu mol\cdot h^{-1}\cdot g^{-1}$, $p<0.001$) and 1000 mU/l insulin (8.45 ± 0.67 vs 11.41 ± 0.44 $\mu mol\cdot h^{-1}\cdot g^{-1}$, $p<0.001$). In order to assess the concentrations of insulin required to stimulate glycolysis half-maximally in the presence of furosemide (EC_{50}, index of sensitivity to insulin), it was important to investigate whether the rates of lactate formation obtained at 10,000 mU/l were maximal. Therefore, rates of lactate formation at insulin concentrations of 10,000 mU/l in the absence or presence of 0.5 and 1.0 mmol/l furosemide were measured (10.24 ± 0.02, 11.27 ± 0.21 and 5.93 ± 0.27 $\mu mol\cdot h^{-1}\cdot g^{-1}$ respectively) and they were not different from those obtained at 10,000 mU/l insulin (10.29 ± 0.05, 13.46 ± 1.30 and 6.63 ± 0.70 $\mu mol\cdot h^{-1}\cdot g^{-1}$ respectively). Therefore, the EC_{50} was calculated for each dose-response curve taking the rates of lactate formation at 10,000 mU/l as maximal. EC_{50} was increased from 92 ± 18 mU/l in control experiments to 303 ± 77 mU/l ($p<0.01$) in the presence of 0.5 mmol/l furosemide, which indicates a decreased sensitivity of this process to insulin (Fig. 1). At a higher concentration of furosemide (1.0 mmol/l) the EC_{50} was similarly increased (209 ± 58 mU/l, $p<0.02$ vs controls), but the rates of glycolysis at all concentrations of insulin were decreased ($p<0.01$ for all values vs controls, Fig. 1). The effects of the drug on the rates of glucose phosphorylation at various insulin concentrations were also studied by calculating the flux from glucose to hexose monophosphates (see Materials and methods section). Identical findings to those described above were obtained, that is, there was no effect of 0.5 mmol/l furosemide at 1, 10 and 10,000 mU/l insulin but the rates of phosphorylation were decreased at 100 and 1000 mU/l insulin (Table 1).

Furosemide (at either 0.5 or 1.0 mmol/l) had no effect on the rates of glycogen synthesis at any insulin concentration. However, 6.0 mmol/l furosemide, inhibited both basal and insulin-stimulated rates of glycogen formation (Table 2).

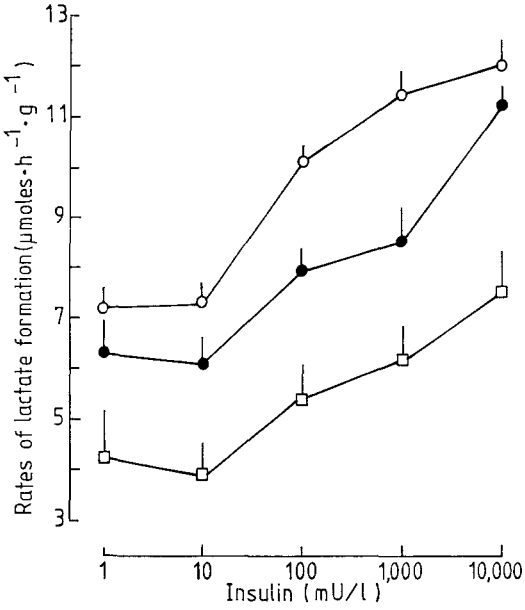

Fig. 1. Basal and insulin-stimulated rates of lactate formation in the soleus muscle of the rat in control experiments (O—O) and following incubation with 0.5 mmol/l (●—●) or 1.0 mmol/l (□—□) furosemide (each point represents mean ± SEM of 10–24 separate incubations)

Table 1. Effects of insulin on rates of glucose phosphorylation by rat soleus muscles in the presence or absence of 0.5 mmol/l furosemide (each point represents the mean ± SEM of 4–13 separate incubations)

Insulin (mU/l)	Flux of glucose to hexose monophosphates ($\mu mol\ glucosyl\cdot h^{-1}\cdot g^{-1}$)	
	Controls	Furosemide
1	5.49 ± 0.19	5.66 ± 0.11
10	5.57 ± 0.43	4.57 ± 0.48
100	7.38 ± 0.39	5.89 ± 0.35^a
1000	10.60 ± 0.51	8.55 ± 0.45^a
10000	11.09 ± 0.54	10.25 ± 0.41

[a] $p<0.05$ vs controls

Table 2. Effects of insulin on rates of [U-^{14}C]-glucose incorporation into glycogen by rat soleus muscles in the presence or absence of furosemide (each point represents the mean ± SEM of 4–10 separate incubations)

Insulin (mU/l)	Glycogen formation ($\mu mol\ glucosyl\cdot h^{-1}\cdot g^{-1}$)			
	Controls	Furosemide		
		0.5 mmol/l	1.0 mmol/l	6.0 mmol/l
1	2.40 ± 0.15	3.00 ± 0.05	3.15 ± 0.25	0.90 ± 0.18^b
10	1.88 ± 0.23	2.01 ± 0.32	1.24 ± 0.20	1.07 ± 0.11^a
100	3.65 ± 0.32	3.03 ± 0.33	3.11 ± 0.30	2.29 ± 0.27^a
1000	5.74 ± 0.28	4.81 ± 0.46	4.88 ± 0.40	3.78 ± 0.19^b
10000	5.94 ± 0.31	6.07 ± 0.40	6.21 ± 0.80	3.62 ± 0.16^c

[a] $p<0.05$
[b] $p<0.01$
[c] $p<0.001$
} vs controls

Discussion

The rates of glycolysis were measured by following the rates of lactate formation which, in this muscle preparation, is the major end-product of glycolysis [17, 18]. Under the conditions of our experiments, the proportion of glucose being oxidised is very small [24]. Furthermore, the major effect of insulin on glucose utilisation in this tissue preparation is on glucose transport which is the flux-generating step for this process [25] and it has been demonstrated that factors that influence the sensitivity of glucose transport to insulin also effect the rate of lactate formation [26].

At a concentration of 0.5 mmol/l, furosemide had no effect on the rates of glycolysis or glucose phosphorylation in isolated incubated rat soleus muscle at basal or maximal concentrations of insulin but it decreased the rates of these processes at 100 and 1000 mU/l insulin. This finding suggests that this drug can decrease the sensitivity of glycolysis to insulin in muscle. The mechanism of this effect is not known but it is of interest that at this concentration, furosemide had no effect on the sensitivity of glycogen synthesis to insulin (Table 2). Furosemide has structural similarities to adenosine [1] so that it may act as an adenosine agonist, which has been shown previously to decrease the sensitivity of glycolysis but to have no effect on the sensitivity of glycogen synthesis to insulin in muscle [27]. In order to investigate this possibility, experiments with adenosine receptor antagonists in the presence or absence of furosemide should be performed. These experiments will be facilitated by the availability of water soluble adenosine receptor antagonists that have been recently synthesised [28].

It should be noted that higher concentrations of furosemide (1.0 mmol/l) inhibited basal glycolytic rates (Fig. 1) and at 6.0 mmol/l inhibited rates of glycogen synthesis (Table 2). These effects may be mediated through direct inhibition of glucose transporters by furosemide as has recently been reported for adipocytes [13].

The results of the present study may have clinical significance. The concentration of furosemide that had an effect on insulin-stimulated glucose utilisation in this study (0.5 mmol/l) is higher than that observed in the serum of patients receiving therapeutic doses of the drug (up to 0.08 mmol/l) [1]. However, even lower concentrations of furosemide will probably have an effect on glycolysis in muscle because the effects at 0.5 mmol/l are already marked. In a recent study in rat adipocytes the effects of furosemide on glucose uptake were shown to be time-dependent, progressive and poorly reversible and incubation of the adipocytes for 48 h at concentrations as low as 0.1 mmol/l furosemide had the same effect as shorter incubations at much higher concentrations of the drug (1.0–6.0 mmol/l) [13]. Although there are no data for muscle, progressive accumulation of furosemide in renal tissue has been reported [29, 30]. It is therefore possible that prolonged administration of furosemide in vivo can lead to progressive accumulation of the drug in muscle and impair the sensitivity of glucose utilisation to insulin by the mechanisms suggested in the present study.

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