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DESENSITIZATION BY TERBUTALINE OF β-ADRENOCEPTORS IN THE GUINEA-PIG SOLEUS MUSCLE: BIOCHEMICAL ALTERATIONS ASSOCIATED WITH FUNCTIONAL CHANGES

T. BUUR, T. CLAUSEN, E. HOLMBERG*, U. JOHANSSON* & B. WALDECK*

Department of Physiology, University of Aarhus, 8000 Århus C, Denmark and Research and Development Laboratories*, AB Draco, Lund, Sweden

1 The effects of adrenaline and terbutaline on cyclic adenosine 3',5'-monophosphate (cyclic AMP) content, ^{22}Na-efflux, ^{42}K-influx and subtetanic contractions have been assessed in soleus muscles isolated from guinea-pigs which had been maintained on food with or without terbutaline for 5 days.

2 Terbutaline and adrenaline increased cyclic AMP content and suppressed subtetanic contractions, and regression analysis indicates a statistically significant correlation between these two effects ($P < 0.01$).

3 In muscles obtained from terbutaline-treated animals, the effects of terbutaline and adrenaline on cyclic AMP content, active Na-K-transport and subtetanic contractions were all considerably suppressed, but insulin stimulated ^{22}Na-efflux and affected subtetanic contractions to the same extent as in the muscles obtained from the control group.

4 The results suggest that terbutaline treatment leads to a reduction in the number of β_2-adrenoceptors in skeletal muscle or an impairment of their function.

5 The results provide further support for the idea that the effect of adrenaline or insulin on skeletal muscle contractions is the outcome of stimulation of active Na-K-transport.

Introduction

The slow-contracting soleus muscle of the guinea-pig responds with a reduced degree of fusion and tension of subtetanic contractions upon stimulation with β-adrenoceptor agonists (for review, see Bowman, 1980). This effect is largely attenuated following five days of treatment with terbutaline, a β_2-selective adrenoceptor agonist (Holmberg, Jeppsson & Waldeck, 1981).

Another well-established effect of β-adrenoceptor agonists on skeletal muscle is the stimulation of the active Na-K-transport across the plasma membrane (Clausen & Flatman, 1977; Rogus, Cheng & Zierler, 1977). Detailed *in vitro* studies on the soleus muscle from guinea-pig and rat, respectively, show that the effects on the contractions (Waldeck, 1977; Al-Jeboory & Marshall, 1978) and on the active Na-K-transport (Clausen & Flatman, 1977; 1980) are both mediated by β_2-adrenoceptors, presumably via stimulation of adenylate cyclase (Clausen & Flatman, 1977; Al-Jeboory & Marshall, 1978; Fellenius, Hedberg, Holmberg & Waldeck, 1980).

In the present study we have examined the effect of treatment with terbutaline *in vivo* on the responsiveness of the guinea-pig soleus muscle to terbutaline and adrenaline *in vitro* with respect to depression of subtetanic contractions, stimulation of the active Na-K-transport and accumulation of 3',5'-cyclic adenosine-monophosphate (cyclic AMP).

Insulin, which stimulates the active Na-K-transport in soleus muscle via a cyclic AMP-independent mechanism (Flatman & Clausen, 1979), causes changes in the contraction pattern of the soleus muscle similar to those produced by β-adrenoceptor agonists (Holmberg & Waldeck, 1980a). It was of interest, therefore, to see if pretreatment with terbutaline would influence the response to insulin.

Methods

All experiments were performed on young fed guinea-pigs (120–200 g). Terbutaline pretreatment was given by maintaining the animals on food containing terbutaline sulphate (100 mg/kg) for 5 days until the night before the experiment. The steady-state level of terbutaline in the plasma during this treatment is in the range 100–130 nmol/l. When the

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animals were killed for the experiments, no terbutaline could be detected in the plasma (Lindberg, personal communication). Animals matched with respect to age and weight were given the same food without terbutaline and served as controls. The animals were killed by decapitation and blood samples collected from the neck vessels into heparinized glass tubes. Following centrifugation, the Na-K-contents of the plasma were determined by flame photometry. Samples of the gastrocnemius muscle were taken for determination of Na-K-contents as described elsewhere (Kohn & Clausen, 1971). Na-efflux was determined by measuring the washout of ^{22}Na from preloaded muscles by methods already developed for rat soleus muscles (Clausen & Kohn, 1977). The Na-efflux was calculated by multiplying the fractional loss of ^{22}Na by the intracellular Na-pool, which was 12 μmol/g wet wt. The ouabain-sensitive component of ^{42}K-influx was measured as described elsewhere (Clausen & Kohn, 1977). Subtetanic contractions and their depression by various agents were recorded by methods described in earlier publications (Waldeck, 1976; Holmberg & Waldeck, 1980b). The tissue content of cyclic AMP was determined with a commercially available assay kit (TRK. 432, The Radiochemical Centre, Amersham). Experimental details are given in the legends to figures and tables.

Results

Measurements of Na-K-contents and Na-efflux

As can be seen from Tables 1 and 2, there was no significant difference between the controls and the treated animals, either with respect to Na-K-content of plasma and muscle, or in basal ^{22}Na-efflux rate or the ouabain-suppressible ^{42}K-uptake.

However, as shown in Figure 1, the effects of terbutaline and adrenaline on the fractional loss of ^{22}Na were considerably suppressed by terbutaline pretreatment. Even at the maximum effective concentration of terbutaline tested (10^{-4} M), the stimulation of ^{22}Na-efflux was diminished by 76% (Figure 1a). The response to adrenaline showed a somewhat smaller, but clearly significant decrease (66% at the concentration (10^{-5} M) producing maximum stimulation).

In accordance with these results, the effect of adrenaline on the ouabain-suppressible (Na-K-pump-mediated) ^{42}K-uptake was virtually abolished in muscles obtained from terbutaline-treated animals (Table 2).

In contrast, the stimulating effect of insulin (25 mu/ml) on ^{22}Na-efflux was not affected by terbutaline pretreatment. The fractional loss of ^{22}Na measured in the presence of insulin was $0.044 \pm 0.003\ min^{-1}$ ($n = 6$) and $0.042 \pm 0.002\ min^{-1}$ ($n = 6$) for the terbutaline pretreated animals and the controls, respectively. This corresponds to 29.6 and 24.4% stimulation in relation to the control level, which was $0.034 \pm 0.001\ min^{-1}$ ($n = 10$). In both instances, this effect was statistically significant ($P < 0.005$).

Measurements of subtetanic contractions and cyclic AMP

The effects of adrenaline and terbutaline on the soleus muscle were measured at two concentrations, one causing maximum or near maximum depression of the subtetanic contractions and one producing

Table 1 Effect of terbutaline pretreatment on Na-K-content of plasma and gastrocnemius muscles and ^{22}Na-efflux from soleus muscles

	Control animals	*Terbutaline-treated animals*	P
Plasma			
Na (mM)	111.1 ± 0.7 (9)	110.8 ± 0.9 (9)	> 0.70
K (mM)	4.2 ± 0.1 (9)	4.3 ± 0.2 (9)	> 0.50
Muscle			
Na (μmol/g wet wt.)	24.4 ± 0.7 (9)	22.2 ± 0.8 (9)	> 0.05
K (μmol/g wet wt.)	91.3 ± 1.8 (9)	94.1 ± 1.8 (9)	> 0.20
^{22}Na-efflux (μmol g^{-1} wet wt. min^{-1}	0.44 ± 0.02 (4)	0.43 ± 0.02 (4)	> 0.60

Guinea-pigs were maintained on food with or without terbutaline sulphate (100 mg/kg) for 5 days until the night before the experiments. Samples of plasma and the gastrocnemius muscle were taken and soleus muscles were prepared for the measurement of ^{22}Na-efflux. The results are given as mean ± s.e. with the number of observations in parentheses.

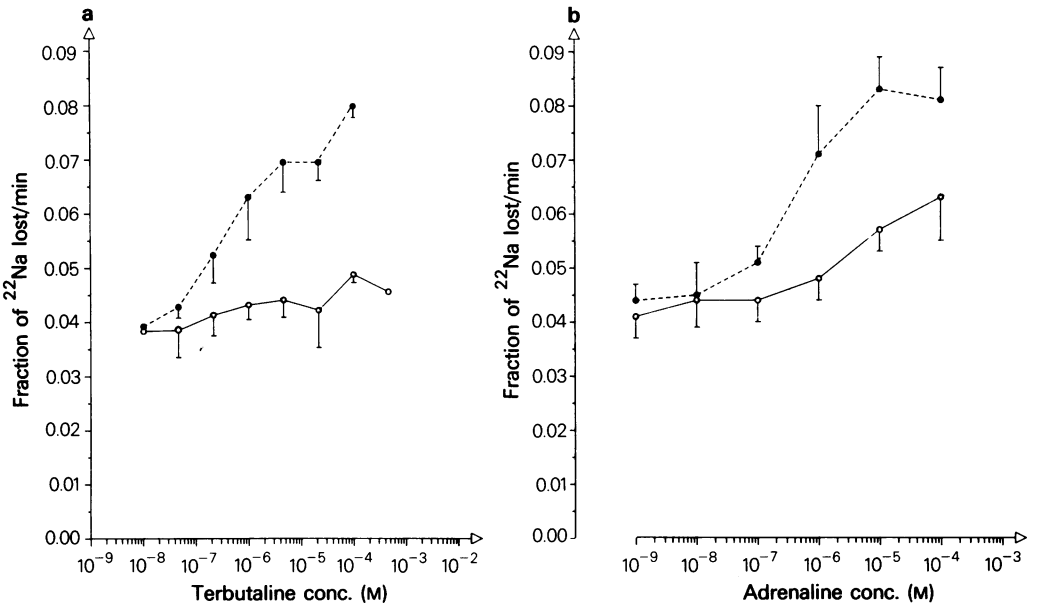

Figure 1 Effect of terbutaline (a) and adrenaline (b) on ^{22}Na-efflux from soleus muscles obtained from guinea-pigs pretreated with terbutaline (——) or control animals (– – –). The muscles were loaded for 90 min by incubation in 3 ml Krebs-Ringer bicarbonate buffer containing 3–5 μCi/ml of ^{22}Na. The muscles were then washed out in a series of tubes containing non-radioactive buffer; 40 min after the start of washout, terbutaline or adrenaline was added to the efflux medium at the concentrations indicated. The mean fractional loss of ^{22}Na as determined from 10 to 20 min after the start of exposure to the drugs is shown. $n = 2-4$ in (a) and 4–5 in (b); vertical lines indicate s.e.mean.

about half maximum response (cf. Waldeck, 1976; 1977). Pretreatment with terbutaline *in vivo* attenuated the maximum response to 0.5 μM adrenaline and to 2.3 μM terbutaline by 80 and 50% respectively ($P<0.001$; Table 3). The response to 0.05 μM adrenaline was completely abolished whereas the effect of 0.22 μM terbutaline was reduced by 70% as compared to the control response ($P<0.001$).

The basal level of cyclic AMP (mean ± s.e. of 12 experiments) in the soleus muscle was 0.69 ± 0.16 pmol/mg dry weight in the control

Table 2 Effect of terbutaline pretreatment on the ouabain-suppressible ^{42}K-influx in guinea-pig soleus muscle

	^{42}K-influx (μmol g^{-1} wet wt. min^{-1}) *Control animals*	*Terbutaline-treated animals*	P
No additions	0.311 ± 0.016 (4)	0.268 ± 0.010 (3)	> 0.05
	$P<0.02$	$P>0.90$	
Adrenaline (10^{-5} M)	0.404 ± 0.024 (4)	0.272 ± 0.037 (4)	< 0.025

Soleus muscles were prepared from terbutaline-treated animals or controls, washed and pre-equilibrated for 15 min with or without ouabain (10^{-3} M) in Krebs-Ringer bicarbonate buffer. They were then transferred into tubes containing ^{42}K (0.1 μCi/ml) incubated for a further 20 min, blotted, weighed and counted. The amount of ^{42}K taken up was calculated on the basis of the specific activity of the incubation medium. The difference between the uptake measured in the presence and the absence of ouabain was taken as the Na-K-pump-mediated component of K-uptake and expressed as mean ± s.e. with the number of observations in parantheses.

Table 3 Effects of terbutaline and adrenaline *in vitro* on the force of contraction of soleus muscles obtained from guinea-pigs pretreated with terbutaline or from control animals

Agonist (μM)	*Subtetanic contractions (% depression)*	
	Control animals	*Terbutaline-treated animals*
Terbutaline, 0.22	25±4 (6)	7±2 (6)
Terbutaline, 2.3	44±3 (6)	21±2 (6)
Adrenaline, 0.05	18±3 (6)	0±1 (6)
Adrenaline, 0.5	35±3 (6)	6±2 (6)

Shown are the means ± s.e. with the number of experiments in parentheses.

group and 0.29±0.15 in the terbutaline-treated group. These values do not differ significantly ($P>0.05$). Adrenaline and terbutaline both increased the level of cyclic AMP in the soleus muscle (Figure 2). This ability was partly lost following treatment with terbutaline for five days. Thus, the response to 0.5 μM adrenaline was diminished by about 60% ($P<0.005$). The increment in cyclic AMP after 2.3 μM terbutaline was 30–40% lower in the treated as compared with the control group ($P<0.05$).

Figure 2 also indicates a correlation between the increase in cyclic AMP following β-adrenoceptor stimulation and the effect on the contractions ($P<0.01$). However, our data do not permit a detailed analysis of this relationship since the cyclic AMP values at the low concentrations of adrenaline and terbutaline were too scattered and have not been included in our results.

In the next series of experiments we measured the effect of insulin *in vitro* on muscles from guinea-pigs treated with terbutaline *in vivo*. Insulin, 25 mu/ml, caused a depression of subtetanic contractions of the soleus muscle qualitatively similar to terbutaline. There was no difference in response between muscles from terbutaline-treated animals and those from controls (Figure 3). In contrast, the response to 2.3 μM terbutaline in the pretreated group was only about 40% of the control ($P<0.025$).

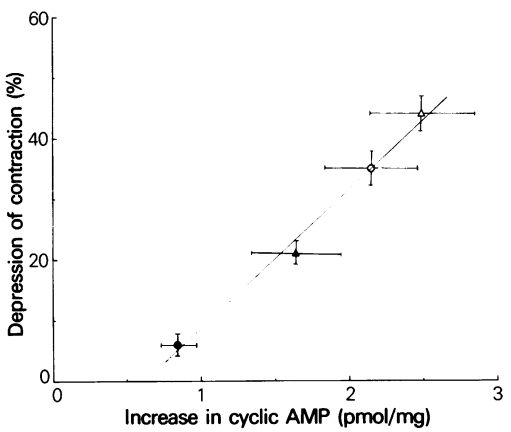

Figure 2 Correlation between the accumulation of cyclic AMP and the depression of subtetanic contractions in the soleus muscle 5 min after the addition of 2.3 μM terbutaline (△) or 0.5 μM adrenaline (○). Open symbols denote muscles from control animals. Filled symbols represent muscles from guinea-pigs pretreated with terbutaline. The results are given as mean values ($n=6$); bars indicate s.e.mean.

Discussion

Treatment with terbutaline *in vivo* for five days resulted in decreased sensitivity of the soleus muscle to terbutaline and adrenaline *in vitro* with respect to (a) depression of subtetanic contractions, (b) accumulation of cyclic AMP and (c) stimulation of the Na-K-pump. These results confirm and extend our previous observations on the development of tolerance to β-adrenoceptor agonists in skeletal muscle (Holmberg *et al.*, 1981).

Refractoriness of β-adrenoceptor-mediated responses to agonists after prolonged stimulation is a well-known phenomenon and it appears to involve a reduction in the number of binding sites (Maguire,

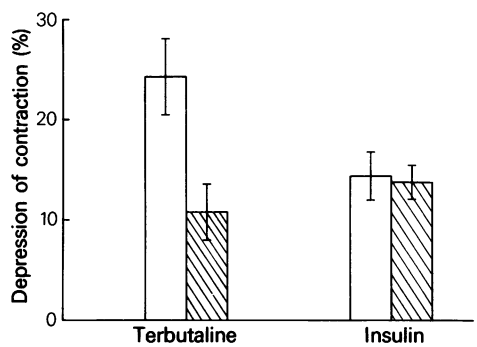

Figure 3 Effect of terbutaline, 2.3 μM, and insulin, 25 mu/ml, subtetanic contractions of soleus muscles obtained from guinea-pigs pretreated with terbutaline (hatched columns) or from control animals (open columns). The results are given as mean values ($n=6$); vertical lines show s.e.mean.

Ross & Gilman, 1977). Thus, the number of β-adrenoceptor binding sites of the soleus muscle of the rat decreased by 40% following 21 days treatment with isoprenaline (Vallières, Cote & Bukowiecki, 1979).

Since in our experiments, even the effect of maximum doses of terbutaline or adrenaline were reduced by pre-exposure to terbutaline *in vivo*, it seems probable that the decreased sensitivity to β-adrenoceptor agonists involves a loss of active binding sites. The reduction in the terbutaline and adrenaline stimulated cyclic AMP accumulation may also involve a change in the coupling between the β-adrenoceptor and the adenylate cyclase and both interpretations have to be left open.

The ability of insulin to depress the contractions (Holmberg & Waldeck, 1980a) and to stimulate the Na-K-pump (Flatman & Clausen, 1979) of the soleus muscle were both unaffected by previous treatment with terbutaline. Propranolol, which blocks the β-adrenoceptors, does not inhibit the effect of insulin on the contractions (Holmberg & Waldeck, 1980a). This shows that the desensitization produced by terbutaline is homologous and concerned with β-adrenoceptors.

The view has previously been expressed (Holmberg & Waldeck, 1980a & b) that the depression by β-adrenoceptor-agonists or insulin of the contractions of the soleus muscle may be secondary to stimulation of the Na-K-pump. The present data do not permit a detailed quantitative comparison of the effects on the Na-K-transport and those on the contractions since they were not obtained from the same muscles. For technical reasons the ionic flux had to be measured in muscles from younger animals than those used for mechanical measurements. However, the results are suggestive of a causal relationship between the stimulation of the Na-K-pump and the depression of the contractions. Alternatively, the two processes may be independent and work in parallel (Rodger & Bowman, 1982). This possibility cannot be ruled out at present.

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