Effects of Clenbuterol and Salbutamol on Tissue Rubidium Uptake In Vivo

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In anesthetized rats, injection of the β_2-adrenoceptor (β_2-AR) agonist clenbuterol (0.45 μmol/kg) caused a marked stimulation of ^{86}RbCl (Rb) uptake by skeletal muscle, but had no effect on other tissues; soleus muscle showed the largest (144% increase) response. Injection of another β_2-AR agonist (salbutamol 0.45 μmol/kg) had no effect on Rb uptake by any tissue except soleus muscle (83%). Both agonists increased body (colonic) temperature to the same extent. A 3-day treatment with salbutamol as a dietary admixture had no effect on body weight, muscle mass, or tissue Rb uptake, whereas the same treatment using clenbuterol produced significant increases in body weight and muscle mass and significant decreases in Rb uptake in three of the four muscle groups studied; Rb uptake in soleus was not affected. In another experiment, the short-term effect of clenbuterol injection on muscle Rb uptake was found to be resistant to a high dose (20 mg/kg) of the selective β_2-AR antagonist ICI 118551. It was concluded that the selective effects of short-term administration of clenbuterol on muscle Rb uptake, coupled with its effects over 3 days on Rb uptake and muscle hypertrophy, implicate β-AR modulation of cation transport (possibly via Na,K-adenosine triphosphatase [ATPase] activity) in the anabolic effects of clenbuterol on muscle protein deposition. Since the stimulation of Rb uptake by clenbuterol was resistant to high doses of a selective β_2-AR antagonist and since salbutamol had little or no effect on muscle hypertrophy or Rb uptake, it is suggested that clenbuterol may exert its effects via an atypical β-AR.

A NOTABLE EFFECT of injections of adrenaline and noradrenaline is a decrease in plasma potassium, although an initial transient hyperkalemia is sometimes observed. The hyperkalemic response is thought to be due to α-adrenoceptor (α-AR) activation of potassium efflux from the liver,[1] but the more obvious and sustained hypokalemic response is believed to be mediated mainly by activation of β_2-adrenoceptors ([β_2-ARs] (see Moratinos and Reverte[2] for review). The evidence for this is based largely on studies describing changes in plasma potassium following administration of selective and nonselective β-AR agonists and antagonists. In vitro studies have mainly concentrated on skeletal muscle, which contains the main pool of potassium in the body, and stimulation of β_2-AR in this tissue is believed to be responsible for the agonist-induced hypokalemia.[2,3] In vitro studies performed in soleus muscle have linked the stimulation of β_2-AR to increased cyclic adenosine monophosphate levels, which in turn lead to activation of Na,K-adenosine triphosphatase (ATPase) activity and the subsequent changes in intracellular potassium and sodium content.[4,5] However, there has been little or no interest in evaluating potassium uptake in vivo to ascertain the relative ability of different tissues to respond to nonselective or selective β-AR agonists.

The tissue selectivity for potassium uptake in response to adrenergic agonists is of interest because long-term treatments with some β_2-AR agonists such as clenbuterol, also produce increases in protein deposition in skeletal muscle, but not in any other major organs or tissues.[6-8] There is evidence for both increases in muscle protein synthesis[7] and decreases in muscle protein degradation[8] during treatment with clenbuterol, but the mechanisms linking β-AR stimulation to either process are unknown. An indirect action of these compounds via some humoral intermediate has been largely ruled out,[9] and a direct effect on the target tissue, skeletal muscle, seems more likely. This suggests either that postreceptor events in skeletal muscle differ from those for β-AR in other tissues or that clenbuterol activates an atypical receptor linked to protein turnover that only appears in skeletal muscle. However, because skeletal muscle plays a dominant role in producing the hypokalemic response to β_2-selective agonists, it is possible that the selective effects of clenbuterol and similar agents on protein accretion in skeletal muscle are related in some way to the activation of Na,K-ATPase and the subsequent changes in intracellular sodium and potassium. In a previous study,[10] this possibility was investigated by long-term treatment of rats with dietary admixtures containing clenbuterol and the Na,K-ATPase inhibitor digoxin. The results showed that the anabolic effects of clenbuterol on gastrocnemius muscle protein deposition were inhibited by digoxin in a dose-dependent manner.

Given this evidence for a possible connection between Na,K-ATPase activity and cation transport in mediating the anabolic effects of clenbuterol, the present study was undertaken to determine potassium uptake by skeletal muscle in vivo following either short- or long-term treatment with clenbuterol. Radioactive-labeled rubidium (Rb) was used as a tracer for potassium, and the tissue accumulation was compared with that obtained after short-term and 3-day treatment with another β_2-AR agonist, salbutamol. Since this was an in vivo study, Rb uptake in other tissues was assessed at the same time and showed that clenbuterol was selective for muscle. The responses to the two agonists differed, and further experiments with clenbuterol and the β_2-selective antagonist ICI 118551 indicated that the effects of clenbuterol on Rb uptake were probably not due to β_2-AR activation.

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MATERIALS AND METHODS

Animals

For the short-term studies, male Wistar rats (140 to 160 g) were obtained from the Biological Research Facility at St George's Hospital Medical School and used without further treatment. Rats weighing 110 to 120 g were used for the 3-day experiment. During the long-term feeding trial, rats were housed in pairs in a room maintained at a temperature of 22° ± 1°C with a controlled light cycle (7 AM to 7 PM) and free access to water. Control animals were fed a powdered stock diet (PRD, Christopher Hill, Dorset, UK), and the experimental groups were fed the same diet containing 2 mg/kg clenbuterol or salbutamol sulfate. Measurements of food intake (corrected for spillage) were recorded each day. At the end of the 3-day feeding period, Rb uptake was measured.

Rb Uptake

Rats were anesthetized with urethane (1.4 g/kg intraperitoneally) and placed under a bench light at a distance sufficient to maintain a normal colonic temperature before injection of agonists, and throughout the experiment in vehicle-injected rats. Colonic temperature was recorded at 15-minute intervals using a rectal thermocouple probe. The left carotid artery was exposed, and a cannula containing heparinized saline was inserted. After a recovery period of 45 to 60 minutes to establish a stable body temperature following surgery, the vehicle (0.9% saline) or 0.45 μmol/kg agonist was injected subcutaneously. Fifteen minutes later, all animals were administered a carotid injection (over 20 seconds) of 250 μL ^{86}Rb (75 μCi/mL) followed by a flushing injection of 200 μL heparinized saline. After 45 minutes, the animals were killed by cervical dislocation. In preliminary experiments, it was found that blood ^{86}Rb radioactivity declined rapidly after injection, reaching a steady low level after approximately 30 minutes. Thus, killing animals 45 minutes after injection of ^{86}Rb (60 minutes after injection of agonist) was considered more than sufficient to allow for any agonist-induced effects on tissue Rb uptake.

Immediately after killing, the soleus and gastrocnemius muscles from one leg and all the muscles from the other leg were dissected and removed as quickly as possible along with interscapular brown adipose tissue (IBAT), back muscle, kidney, liver, diaphragm, and heart, always in the same order. After weighing, radioactivity of the tissue samples was measured (5-minute counts) in a gamma counter (Beckman Gamma 5500, Beckman Instruments, Fullerton, CA). To measure the radioactivity injected, 300 μL of the ^{86}RbCl solution was placed in a vial and counted. After removing and injecting the animal with 250 μL, the vial was recounted and the counts (counts per minute) removed were corrected for those in a second vial containing residual radioactivity flushed from the syringe used for injection. Differences in counts injected per gram rat were not significant between groups in any experiment, and therefore, the results have been expressed as counts per minute per milligram wet tissue weight.

In the first experiment, short-term effects of clenbuterol and salbutamol on Rb uptake were compared using equimolar doses shown to be isothermogenic (same increase in colonic temperature) in preliminary tests. In the second experiment, effects of the two agonists were compared over 3 days using a level (2 mg/kg diet) that has been used in many other previous studies on the anabolic effects of clenbuterol (see Yang and McElligott[9] for a review). In the final experiment, the effect of the selective β_2-antagonist ICI 118551 alone or in combination with clenbuterol was assessed by injecting the antagonist (20 mg/kg intraperitoneally) 10 minutes before injection of clenbuterol or vehicle. The dose of ICI 118551 was a supramaximal dose, and compares with doses as low as 30 μg/kg that block or reduce the effect of other β_2-AR agonists on plasma potassium.[11]

Materials

Radiolabeled ^{86}RbCl was purchased from Amersham International (Amersham, UK), and salbutamol and salbutamol sulfate were purchased from Sigma (Poole, UK). Clenbuterol and ICI 118551 were gifts from Boehringer Ingleheim (Bracknell, UK) and ICI Pharmaceuticals (Macclesfield, UK), respectively. Drugs for injection were dissolved in saline except for ICI 118551, which was dissolved in 1 mL dimethyl sulfoxide and brought to a final volume of 10 mL with water.

Statistics

Results are expressed as the mean ± SEM. Data were subjected to ANOVA, and specific comparisons of differences between control and experimental groups were made using Student's *t* test for unmatched data. When appropriate, ANOVA followed by Scheffé's test was used. All probabilities quoted are two-tailed.

RESULTS

Clenbuterol treatment caused a marked (68% to 145%) increase in skeletal muscle Rb uptake as compared with that of vehicle-treated controls (Table 1), but had no effect on Rb uptake in the other tissues examined. When comparing individual muscles, the response to clenbuterol in soleus was found to be much greater (145%) than the responses (70% on average) in the other muscles studied. Apart from soleus muscle (83% increase), salbutamol failed to produce an increase in Rb uptake in any of the other tissues. However, salbutamol produced a significant increase in body (colonic) temperature, similar to that produced by clenbuterol.

Short-term (3-day) pretreatment with clenbuterol or salbutamol had no effect on food intake (19 to 20 g/d in all groups), but clenbuterol produced a significant increase in body weight gain and soleus and gastrocnemius muscle mass (Table 2). Treatment with salbutamol for the same period of time had no effect on weight gain or muscle

Table 1. Short-Term Effects of Clenbuterol and Salbutamol on Tissue ^{86}Rb Uptake and Body (colonic) Temperature

	Control	Clenbuterol	Salbutamol
Soleus	14.9 ± 2.2[a]	36.3 ± 2.7[b]	27.2 ± 2.7[b]
Gastrocnemius	15.9 ± 2.0[a]	27.2 ± 1.2[b]	22.7 ± 1.7[ab]
Whole leg	14.5 ± 1.9[a]	24.3 ± 1.4[b]	18.2 ± 1.3[ab]
Back muscle	15.6 ± 1.8[a]	26.3 ± 1.5[b]	22.3 ± 1.8[ab]
Diaphragm	44.3 ± 3.7	51.3 ± 6.5	53.1 ± 4.1
Heart	36.8 ± 2.0	41.3 ± 2.5	45.7 ± 6.2
Liver	54.9 ± 5.4	50.5 ± 3.2	51.8 ± 5.4
Kidney	51.4 ± 4.5	48.9 ± 4.0	45.6 ± 2.6
IBAT	33.7 ± 5.8	33.3 ± 5.3	30.5 ± 3.7
dT	0.0 ± 0.1[a]	0.9 ± 0.1[b]	0.7 ± 0.2[b]

NOTE. Results are expressed as cpm/mg wet tissue weight and are the mean ± SEM (n = 6). Values sharing the same superscript are not significantly different ($P > .05$, Scheffé's test).

Abbreviation: dT, increase in colonic temperature (°C) above pre-injection level.

weight (Table 2). Rb uptake measurements could not be made on all rats at the same time, and so the 3-day feeding treatments were staggered, which meant using separate control groups for the two drug-treated groups. Three days of salbutamol pretreatment had no effect on Rb uptake (Table 3), whereas in contrast to its short-term effects, 3 days of clenbuterol treatment inhibited uptake significantly in three of the four muscles studied. The exception was soleus, in which uptake was no different from control uptake. Three days of treatment caused a 24% increase in Rb uptake by diaphragm, which compares with the 16% (nonsignificant) increase produced by short-term treatment.

In the absence of any agonist, treatment with the β_2-AR antagonist ICI 118551 by itself produced changes in Rb uptake (Table 4), but apart from IBAT, this effect occurred only in those tissues not affected by short-term clenbuterol agonist treatment in the first experiment. Rb uptake in skeletal muscle and IBAT was unaffected, but uptake by diaphragm, heart, liver, and kidney in the presence of ICI 118551 was significantly increased by 40% to 90% as compared with control values. In addition to these effects of the antagonist itself, ICI 118551 also produced divergent changes in the response to clenbuterol. For example, the combined effects of the antagonist plus clenbuterol resulted in increased Rb uptake in IBAT, whereas neither the antagonist alone (Table 4) nor the agonist alone (Table 1) affected IBAT. This compares with the kidney, where treatment with antagonist alone stimulated uptake and combined treatment with clenbuterol decreased Rb uptake, whereas clenbuterol treatment alone (Table 1) failed to have any effect on Rb uptake by the kidney.

In contrast to these responses, the effect of clenbuterol on Rb uptake by the various leg and back muscles appeared resistant to β_2-AR blockade with ICI 118551, and the agonist was still capable of producing significant 30% to 114% increases as compared with Rb uptake with ICI 118551 alone or with control values. In fact, values for Rb uptake during β_2-AR blockade were approximately equivalent to 80% to 90% of those seen in the first experiment (Table 1), which was conducted under identical conditions. ICI 118551 had no effect on colonic temperature by itself, but appeared to potentiate the clenbuterol response, since the increase in body temperature was 44% greater than that induced by the agonist alone in the first experiment.

DISCUSSION

Unlike most previous studies where plasma potassium changes have been monitored, the approach used here to investigate the effects of β-AR activation on potassium handling was to measure tissue uptake in vivo using ^{86}Rb as a labeled tracer. ^{86}Rb was chosen instead of ^{42}K, since for various reasons (half-life, etc.), ^{86}Rb is experimentally easier to deal with. In the experiments described, the radioactivity present in the tissues was measured 45 minutes after injection of the tracer, and like Struthers et al,[12] who used ^{42}K to investigate the variations in uptake during adrenaline infusion, it was assumed that differences in tissue radioactivity after this short exposure represented differences in Rb uptake—ie, Rb efflux was negligible (subsequent unpublished experiments on muscle Rb efflux in vitro have shown no effect of clenbuterol).

Using this in vivo approach, it was possible to show that the tissue selectivity for the effects of clenbuterol on Rb uptake corresponded to its selective anabolic effects on skeletal muscle—ie, Rb uptake was increased only in the leg and back muscles and not in any other tissue examined. Salbutamol had a much weaker effect on soleus (83% stimulation) than clenbuterol (145% stimulation) and did not affect Rb uptake in any of the other muscles studied. This difference in potency between the effects of the two β_2-AR agonists on Rb uptake correlates with reports that salbutamol has little or no anabolic actions,[13] and this was confirmed by the subchronic (3-day) experiment reported here. However, it could be argued that differences in metabolic stability may result in artifactual differences in potency between the two drugs, particularly since clenbuterol was developed as a long-acting β_2-agonist. Nevertheless, such a difference would not explain the observation that the thermogenic response (ie, increase in colonic temperature) during the Rb uptake experiments was the same for clenbuterol and salbutamol, suggesting that the thermogenic potency and duration were equivalent, at least in the short-term experiments. Furthermore, the anabolic

Table 2. Body Weight Gain, Food Intake, and Tissue Weights After 3-Day Pretreatment With Clenbuterol and Salbutamol

	Control (n = 12)	Clenbuterol (n = 6)	Salbutamol (n = 6)
Food intake (g)	58 ± 3	62 ± 4	60 ± 3
Weight gain (g)	24 ± 1	32 ± 3*	23 ± 1
Soleus (mg)	52 ± 1	58 ± 1*	51 ± 2
Gastrocnemius (mg)	656 ± 12	714 ± 13*	662 ± 13
Heart (mg)	420 ± 15	435 ± 10	445 ± 11

NOTE. Results are the mean ± SEM. Control values are pooled from separate clenbuterol and salbutamol experiments.

*$P < .01$ *v* control group (*t* test).

Table 3. ^{86}Rb Uptake After 3-Day Pretreatment With Salbutamol and Clenbuterol

	Control	Salbutamol	Control	Clenbuterol
Soleus	20.3 ± 2.4	24.7 ± 1.1	19.6 ± 1.2	22.0 ± 1.9
Gastrocnemius	26.1 ± 2.5	27.0 ± 1.6	21.0 ± 1.5	16.3 ± 1.4*
Whole leg	23.8 ± 3.6	25.5 ± 1.0	20.0 ± 1.8	13.7 ± 1.7†
Back muscle	31.2 ± 1.4	30.4 ± 3.5	21.4 ± 1.3	14.8 ± 1.3†
Diaphragm	58.7 ± 2.9	56.7 ± 3.6	43.9 ± 1.3	54.5 ± 2.4†
Heart	56.6 ± 2.4	54.4 ± 2.1	40.7 ± 1.7	44.2 ± 2.9
Liver	87.0 ± 2.1	90.9 ± 4.8	70.8 ± 6.0	79.2 ± 8.4
Kidney	78.9 ± 7.4	75.6 ± 5.3	55.4 ± 2.1	61.8 ± 4.9
IBAT	37.7 ± 6.1	35.2 ± 3.0	30.7 ± 3.0	36.6 ± 4.6

NOTE. Results are expressed as cpm/mg wet tissue weight and are the mean ± SEM (n = 6).
*$P < .05$, †$P < .01$: *v* respective control group (unpaired *t* test).

effects of clenbuterol may relate more to its ability and greater potency in activating an atypical β-AR (see below) than to its greater duration of action. To assist the next part of the discussion, the Rb uptake results obtained in tissues other than skeletal muscle have been presented in Fig 1, where the effects of clenbuterol, ICI 118551, and their co-administration have been presented as a percentage of control values.

Adrenergic activation of thermogenesis is now thought to be mainly due to stimulation of BAT β_3-AR,[14,15] and the fact that some β_1-AR and β_2-AR agonists have a degree of thermogenic activity[16] implies either a loss of agonist selectivity or some β_1/β_2-AR involvement in thermogenesis. Whatever the reason, the thermogenic (ie, colonic temperature) responses to clenbuterol and salbutamol observed in these experiments would have been expected to be associated with increases in IBAT Rb uptake, since BAT thermogenesis is normally accompanied by increased Na,K-ATPase activity.[17] It would be tempting to suggest that BAT was completely unaffected by the two β_2-AR agonists, and that the increases in body temperature originated elsewhere. However, this cannot be reconciled with the observation that clenbuterol in the presence of ICI 118551 caused a marked increase in IBAT Rb uptake (Table 4, Fig 1). If this increase in Rb uptake was associated with activation of BAT thermogenesis, it could explain why the colonic temperature response to clenbuterol was potentiated by ICI 118551 (Fig 1). Thermal responses to the nonselective β-AR agonist isoprenaline can also be potentiated by inhibition with ICI 118551 of its β_2-AR–mediated actions,[18] and the fact that both the thermogenic activity of clenbuterol is enhanced and its effects on cation transport in IBAT are unmasked by ICI 118551 suggests that its actions are not restricted to the β_2-AR.

Table 4. Effects of ICI 118551 on Tissue ^{86}Rb Uptake and Body (colonic) Temperature

	Control	ICI 118551	ICI 118551 + Clenbuterol
Soleus	13.8 ± 2.1^a	18.8 ± 1.1^a	29.6 ± 1.1^b
Gastrocnemius	14.5 ± 1.2^a	14.2 ± 1.1^a	23.1 ± 1.3^b
Whole leg	14.1 ± 1.7^a	14.5 ± 0.9^a	23.7 ± 2.5^b
Back muscle	15.2 ± 1.5^a	16.0 ± 0.9^{ab}	20.7 ± 1.1^b
Diaphragm	42.4 ± 2.6^a	59.9 ± 2.5^b	70.9 ± 4.3^b
Heart	36.1 ± 1.7^a	64.2 ± 2.3^b	58.9 ± 2.4^b
Liver	50.8 ± 5.6^a	89.6 ± 3.2^b	89.5 ± 3.8^b
Kidney	48.7 ± 4.1^a	91.7 ± 3.2^b	69.9 ± 4.3^c
IBAT	35.3 ± 5.5^a	35.5 ± 3.5^a	65.4 ± 5.7^b
dT	0.0 ± 0.1^a	0.1 ± 0.1^a	1.3 ± 0.2^b

NOTE. Results are expressed as cpm/mg wet tissue weight and are the mean ± SEM (n = 6). Values sharing the same superscript are not significantly different ($P > .05$, Scheffé's test).

Abbreviation: dT, increase in colonic temperature (°C) above pre-injection level.

Without further information, it is clearly not possible to resolve these paradoxical effects of clenbuterol and ICI 118551 on Rb uptake by IBAT, and the results obtained with ICI 118551 also raise questions about the adrenergic control of Rb uptake in some of the other tissues studied. For example, the antagonist alone appeared to stimulate uptake in diaphragm, heart, liver, and kidney (Table 4, Fig 1), which might suggest that there is some endogenous β_2-inhibitory influence on Rb (ie, potassium) uptake in these tissues. However, as with BAT, this would not be consistent with the observation that neither of the two β_2-AR agonists (clenbuterol or salbutamol) affected Rb uptake in either direction in these tissues. An alternative explanation relies on the observation that ICI 118551 can cause release of noradrenaline,[19] but this would not explain why the antagonist failed to affect Rb uptake in skeletal muscle and IBAT and had no effect on body temperature. Another possibility is that ICI 118551 blocked endogenous vasodilatation in peripheral tissues and diverted blood flow to the heart, liver, and kidney. The kidney results are particularly puzzling (Fig 1), since clenbuterol had no effect on its own but in the presence of ICI 118551 inhibited the increased Rb uptake induced by the β_2-AR antagonist. One possible explanation is that this combination of drugs was interfering with the β-AR–mediated antikaliuretic mechanism in the collecting ducts.[20]

The observation that the effects of clenbuterol on muscle were resistant to the β_2-antagonist, with the agonist still producing large (30% to 60%) and significant increases in Rb uptake in the presence of a high dose of ICI 118551, would be consistent with the involvement of an atypical β-AR. As mentioned in the Methods, the dose of ICI 118551 used (20 mg/kg) was much greater (up to 600 times) than that required to block other β_2-AR–mediated effects. A comparison of β_2-AR affinity with biological potency

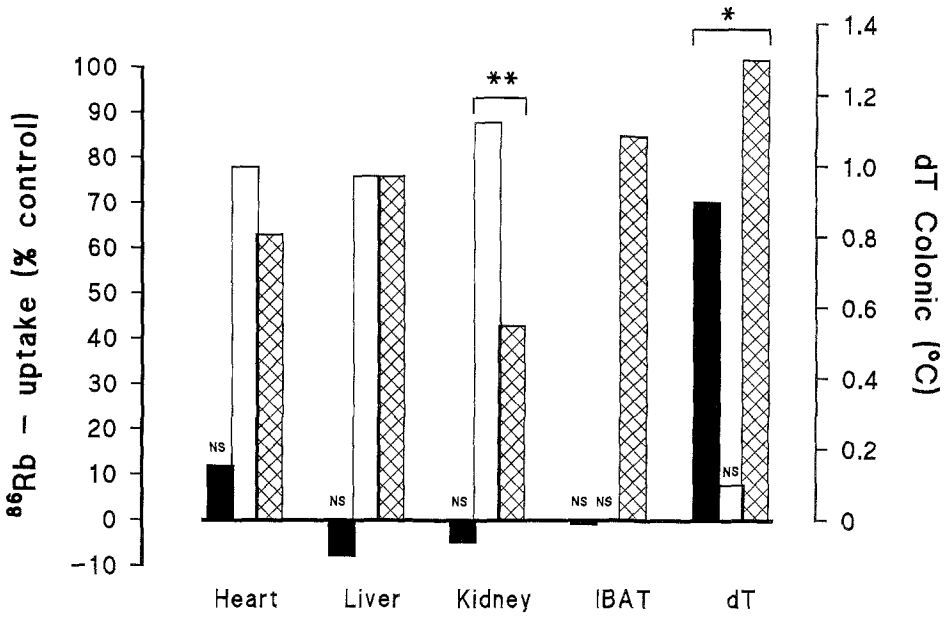

Fig 1. Effects of (■) clenbuterol, (□) ICI 118551, and (▨) combined treatment on Rb uptake (% control value) and body temperature (dT colonic). Changes are calculated from data in Tables 1 and 4, and only nonsignificant (NS = $P > .05$, Scheffé's test) changes relative to control Rb uptake have been indicated in the figure, apart from $*P < .05$, $**P < .01$ for unpaired t test comparisons.

(ED_{50} for inhibition of urinary nitrogen excretion) of clenbuterol and five analogs[21] provides further evidence for clenbuterol's possessing some atypical β-AR activity. This would be consistent with ligand displacement studies on rat sarcolemma membranes,[22-24] which demonstrate that muscle possesses an atypical β-AR. Interestingly, the muscle that appears to have the greatest density of atypical β-AR (over 80% of total β-AR[24]; and unpublished data of D.K. Sudera and M.J. Stock, January 1994) is soleus, which is also the muscle that shows the greatest anabolic and Rb uptake response to clenbuterol. If clenbuterol does exert a significant effect on muscle via an atypical AR, it is tempting to implicate the β_3-AR originally identified in adipose tissue[14,15] but also implicated in β-AR metabolic responses in muscle.[25] Furthermore, there is evidence that two of the selective β_3-AR agonists (BRL 37344 and SR 58611A) produce a hypokalemia that is resistant to conventional β-AR antagonists (eg, ICI 118551), thereby implicating an atypical β-AR.[26] However, the fact that the selective β_3-AR agonists tested so far have been reported to have little or no anabolic effects on muscle,[25] suggests that if clenbuterol acts on muscle via an atypical AR, it may not be the same as the adipocyte β_3-AR. Arch et al[25] have suggested the term "atypical β_2-AR" as a more appropriate designation.

Whatever AR subtype is responsible for the selective effects of clenbuterol on muscle Rb uptake, it seems more than likely that the mechanism involves activation of Na,K-ATPase. Increased sodium-pump activity has been linked to the hypokalemic effects of other β-AR agonists,[5,12,27,28] and the activation of Na,K-ATPase is probably due to increased intracellular cyclic adenosine monophosphate levels following β-AR stimulation.[4] The effects of clenbuterol on Rb uptake could be due to a similar mechanism, since MacLennan and Edwards[29] have shown that a single injection of clenbuterol produces a doubling of muscle cyclic adenosine monophosphate levels that persists for at least 5 hours, and inhibition of Na,K-ATPase with digoxin inhibits the anabolic effects of clenbuterol.[10] Apart from activation of Na,K-ATPase, another possible influence on Rb uptake is tissue perfusion. In an earlier study from this laboratory,[30] short-term treatment with clenbuterol increased the skeletal muscle blood flow rate by 75% to 150%. However, it also increased blood flow to IBAT and diaphragm and decreased blood flow to kidney—changes that are not consistent with those observed for Rb uptake in the same tissues in the present study. This vitiates any connection between the effects of the agonist on tissue blood flow and Rb uptake. However, there were some similarities between the two studies, and the reduction in muscle blood flow following long-term treatment with clenbuterol observed in the earlier study[30] is analogous to the inhibition of muscle Rb uptake observed in the 3-day subchronic experiment described here—ie, in both studies, the longer-term effects of clenbuterol were the opposite of its short-term effects. These decreased responses may be a reflection of the receptor downregulation observed following long-term clenbuterol treatment,[30] or of reduced Na,K-ATPase activity, as seen in skeletal muscle after long-term β-agonist treatment.[11] Kim and Sainz[31] have shown that the increase in muscle weight with a similar agonist (cimaterol) occurs over the first 7 days of treatment with no further increase above control rates thereafter, and this coincides with the time at which β-AR density reaches its minimum level. This could explain the reduction in muscle Rb uptake in animals treated for 3 days with clenbuterol.

Overall, these results provide in vivo evidence to support the suggestion[12,32,33] that the hypokalemic effects of β_2-agonists are mainly due to stimulation of potassium uptake by skeletal muscles, particularly slow-twitch working muscles such as soleus. The fact that the stimulation of Rb uptake by clenbuterol was confined to muscle and that it was more potent than that produced by salbutamol correlates closely

with its relative potency and selectivity in stimulating protein deposition in muscle. The observation that, unlike clenbuterol, 3-day treatment with salbutamol had no effect on body weight, muscle mass, or Rb uptake lends further support to the notion of linking the effects of clenbuterol on muscle cation exchange with its anabolic actions. The intracellular cation concentration may be involved in muscle protein deposition, since intracellular sodium affects myofibrillar protein degradation in rat skeletal muscle[34] and since protein synthesis in vivo,[35] in intact cells,[36] and in cell-free systems[37] is highly potassium-dependent. It is also possible that the increases in intracellular volume that would accompany potassium accumulation could also activate anabolic pathways.[38] The suggestion that clenbuterol exerts its long-term anabolic effects on muscle protein deposition by stimulating these pathways via an atypical AR linked to Na,K-ATPase activity warrants further investigation.

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