Cardiovascular Effects of Clenbuterol are \( \beta_2 \)-Adrenoceptor-Mediated in Steers

A. J. Hoey, M. L. Matthews, T. W. Badran, G. G. Pegg, and M. N. Sillence

Tropical Beef Centre: A Joint Venture Between the Central Queensland University, CSIRO Division of Tropical Animal Production, and Queensland Department of Primary Industries, Rockhampton, Queensland, 4702, Australia

ABSTRACT: The mechanism through which the repartitioning agent clenbuterol increases heart rate was investigated. First, the relative importance of the \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors was established in rat and bovine right atria in vitro. The positive chronotropic and inotropic effects of \((\pm)\)isoproterenol in rat and bovine right atria, respectively, were markedly antagonized \((P < .001)\) by the \( \beta_1 \)-adrenoceptor antagonist CGP 20712A but were antagonized less by the \( \beta_2 \)-adrenoceptor antagonist ICI 118 551 in rat \((P < .01)\), but not in bovine atria, indicating a major role of the \( \beta_1 \)-adrenoceptors. Clenbuterol was only a partial agonist in rat right atria, increasing heart rate at high concentrations through stimulation of \( \beta_1 \)-adrenoceptors. In studies in vivo, clenbuterol decreased the plasma potassium concentration \((P < .05)\) and increased the plasma glucose concentration \((P < .05)\). Clenbuterol also reduced diastolic blood pressure \((P < .01)\) and increased heart rate \((P < .001)\). The increase in heart rate was not due to direct stimulation of cardiac \( \beta_1 \)-adrenoceptors by clenbuterol but was consistent with a reflex response to \( \beta_2 \)-adrenoceptor-mediated hypotension. This would have caused the activation of baroreceptors, which in turn would have resulted in both the release of norepinephrine to stimulate cardiac \( \beta_1 \)-adrenoceptors and the inhibition of cholinergic input to the heart. Thus, the effects of clenbuterol could be eliminated completely by ICI 118 551 or reduced by approximately 50% using CGP 20712A. The combination of treatment of clenbuterol and CGP 20712A could be useful. It may allow the full repartitioning effects seen with the \( \beta_2 \)-agonist alone, but with a markedly attenuated effect on the heart. Such a treatment regimen may also help reduce the increased energy expenditure and loss of appetite seen following the initial administration of clenbuterol.

Key Words: Clenbuterol, \( \beta \)-Adrenergic Agonists, \( \beta \)-Adrenergic Receptors, Cardiovascular System, Heart Rate, Cattle

Introduction

Interest in the use of \( \beta \)-adrenoceptor agonists (\( \beta \)-agonists) in animal production has been generated by the discovery that increased carcass and muscle weights are evident in several species of animals fed these compounds (Ricks et al., 1984; MacRae et al., 1988; Sillence et al., 1993). In particular, the long-acting \( \beta_2 \)-adrenoceptor-selective compound clenbuterol (CLEN) has been investigated. It has been noted that apart from the effects of CLEN on skeletal muscle, this compound also increases heart rate and blood flow for the first few days of treatment (Brockway et al., 1987; Eisemann et al., 1988), an effect that subsides with continued administration (Williams et al., 1986, Eisemann et al., 1988; Bruckmaier and Blum, 1992). It is thought that these cardiovascular effects are probably due to a lack of selectivity of CLEN for the \( \beta_2 \)-adrenoceptor (Bruckmaier and Blum, 1992); the drug is also known to stimulate \( \beta_1 \)-adrenoceptors that predominate in the rat heart (Cohen et al., 1982) and in the hearts of most other species. However, \( \beta_2 \)-agonists cause relaxation of vascular smooth muscle and a consequent fall in blood pressure. Thus, an additional or alternative cause of the increase in heart rate could be a reflex action to counteract the \( \beta_2 \)-adrenoceptor-mediated hypotension. Finally, a direct effect of CLEN on cardiac \( \beta_2 \)-adrenoceptors is possible; this subtype has been shown in rat heart to play a minor role in the chronotropic response to adrenaline (Kaumann, 1986). An understanding of the relative importance that \( \beta_1 \)- and \( \beta_2 \)-adrenoceptors play in...
control of heart rate is essential in determining how β-agonists increase heart rate and in establishing whether this adverse effect can be eliminated by designing repartitioning agents that have less activity at the β1-adrenoceptors. In this study there were two aims: first, to examine the importance, with respect to increases in heart rate, of the β1- and β2-adrenoceptors in the right atria of rats and cattle and, second, to examine the effects of CLEN on the cardiovascular system of rats and steers to determine the exact mechanism through which β2-agonists increase heart rate.

**Materials and Methods**

**Rat Right Atrial Rate**

Male Wistar rats (250 to 300 g), obtained from the Central Animal Breeding Laboratories, The University of Queensland, were used to determine the effects of CLEN on right atrial rate. Rats were stunned by a blow to the head and killed by cervical dislocation and exsanguination. The heart was rapidly removed and placed in Tyrode solution (136.9 mM NaCl, 5.4 mM KCl, 1.05 mM MgCl2·H2O, 42 mM NaH2PO4·2H2O, 22.6 mM NaHCO3, 1.8 mM CaCl2, 5.5 mM glucose, .28 mM ascorbic acid). The right atrium from each rat was dissected free with the pacemaker intact and suspended in 25-mL water-jacketed organ bath (35 ± 1°C) filled with Tyrode solution and continuously aerated with carbogen (95% O2, 5% CO2), thus allowing it to beat spontaneously. Each atrium was allowed to stabilize for 90 min with regular washes during the first half of this period. The tissues were then washed by several exchanges of the Tyrode solution.

**Experiments with Isolated Bovine Right Atria**

Three Brahman-cross steers (140 to 158 kg) were killed using a captive-bolt pistol and exsanguinated. The heart was removed and the right atrium dissected free and placed in Tyrode solution stored on ice. The right atrium was carefully opened in Tyrode solution and free-running trabeculae, approximately 8 to 10 mm in length and 1 to 1.5 mm diameter (20.0 ± 1.9 mg, n = 12), were dissected free. These were suspended in organ baths by a clamp at one end and connected to a force transducer by a silk thread at the other end. The muscle was stimulated electrically via platinum electrodes on either side of the clamp (stimulation frequency 1.0 Hz, stimulation strength 20% above threshold, pulse width .1 ms; SD9 stimulator, Grass, Quincy, MA). For the remainder of the experiment the same protocol as that already described for the experiments using rat right atria was used.

**Catheterization of Steers**

Four Brahman-cross steers (range 100 to 110 kg), which were trained to stand quietly while being handled, were housed individually in pens and maintained on 5 kg/d of alfalfa hay, and water ad libitum. Feed was withheld for 24 h before catheterization. Rapid anesthesia was induced by an intrajugular infusion of thiopentone sodium (11 mg/kg) and anesthesia was maintained by an intramuscular injection of xylazine (1 mg/kg). Each steer was laid in a recumbent position on an operating table. An incision was made in the hind limb just above the saphenous artery, and an indwelling catheter was carefully fed into the artery to a predetermined length (approximately 70 cm), which resulted in the positioning of the tip of the catheter in the descending aorta. The vessel was then tied off around the sleeve of the catheter and filled with heparinized saline before the skin was sutured together again. The catheter was plugged and kept in a cloth pouch fixed to the skin using adhesive. Each steer was administered antibiotics (20 mL of penicillin G procaine [250 mg/mL] and procaine hydrochloride [20 mg/mL] in dihydrostreptomycin sulphate solution [250 mg/mL]) and allowed 3 d to recover from the surgery. The day before the initial infusion, a catheter was placed into the jugular vein of each steer.

**Infusion Protocol**

Each infusion was performed on a separate day. The steer was placed in a crush (squeeze chute) and a heart rate monitor was fitted (HR-8AE, Respironics, Hong Kong). Systemic blood pressure was recorded by connecting the arterial catheter to a blood pressure transducer with a digital display (model 3301, Kontron Medical, Munchenstein, Switzerland). Heart rate and blood pressure were recorded every 5 min. Venous
blood samples (2 x 2 mL) were obtained from the jugular catheter every 10 min. The first 2 mL was discarded and the second 2 mL was stored on ice. The catheter was flushed with heparinized saline after sampling each time. Blood samples were subsequently centrifuged to obtain plasma samples that were stored at -80°C until they were analyzed.

After 40 to 60 min of baseline recording, 500 μg of CLEN, diluted in 5 mL of sterile saline, was infused into the jugular vein over a period of 1 min. If the heart rate did not increase by approximately 50 bpm over the subsequent 20 min, then additional CLEN was infused in 250-μg doses up to a total dose of 1 mg. After the heart rate was stable for at least four consecutive readings (20 min), one of two protocols was conducted to compare the ability of two β-adrenoceptor-subtype-selective antagonists to reverse the effects of CLEN. In Protocol 1, 5 mg of the β1-adrenoceptor-selective antagonist CGP 20712A was infused in an attempt to reduce heart rate. After the heart rate had stabilized for at least four consecutive readings, a further 5 mg of CGP 20712A was infused. After the heart rate had stabilized for at least four consecutive readings and no further reduction was achieved in response to the increased dose of CGP 20712A, then 5 mg of the β2-adrenoceptor-selective antagonist ICI 118 551 was infused to cause a reduction in heart rate. After the heart rate stabilized for at least four consecutive readings, an additional 5 mg of ICI 118 551 was infused to determine whether any further reduction in heart rate could be achieved.

In Protocol 2, 5 mg of ICI 118 551 was infused to cause a reduction in heart rate. After the heart rate stabilized for at least four consecutive readings, an additional 5 mg of ICI 118 551 was infused to determine whether any further reduction in heart rate could be achieved. On two occasions this last step was repeated until the steers received a total of 15 and 20 mg of ICI 118 551, respectively. In this way, the maximum effect of ICI 118 551 was determined.

The plasma glucose concentration was measured by reading the absorbance at 505 nm of 100 μL of plasma diluted in 3 mL of glucose (Trinder) reagent (Sigma Diagnostics glucose reagent, Sigma Chemical, St. Louis, MO) and allowed to stand at room temperature for 18 min. Sigma glucose/urea nitrogen combined standards were used for calibration purposes. The plasma K+ concentration was measured by flame photometry.

Each steer was treated according to both Protocols 1 and 2 with at least 4 d between experiments to allow the drugs to be cleared from the steers. Two steers were treated with Protocol 1 first and two steers were treated with Protocol 2 first. At the completion of both protocols, the steers were left for several weeks, again to allow the clearance of all drugs, before being killed for the organ bath experiments described above.

**Drugs**

Clenbuterol hydrochloride was synthesized in our laboratory as described by Pegg et al. (1991). The drug was dissolved in a small volume of dimethyl sulfoxide and then diluted with sterile saline (9%) to a concentration of 100 μg/mL for use in experiments in vivo, or in demineralized water to a range from 100 mM to 1 μM for organ bath experiments. The ICI 118 551 was synthesized in our laboratory by a modification of the method described by Hutton (1989) and was dissolved in dimethyl sulfoxide. The CGP 20712A was obtained from Ciba-Geigy and was dissolved in acidified demineralized water. These antagonists were further diluted with sterile saline (9%) to 2 mg/mL for use in experiments in vivo, or with demineralized water to a range from 1 mM to 100 μM for organ bath experiments. (±)isoproterenol was obtained from Sigma Chemical (St. Louis, MO) and dissolved in acidified demineralized water in a concentration range from 0.1 μM to 10 mM. All other chemicals were laboratory grade.

**Statistics**

Student’s t-test for paired data was used to determine statistical significance. Means were considered significantly different when P < .05. In all cases each tissue or each animal was used as its own control unless otherwise specified. Data are presented as mean ± SE.

**Animal Ethics**

All experiments were performed with the approval of the Animal Experimentation Ethics Committee of the Tropical Beef Centre, Rockhampton, Australia.

**Results**

**Right Atrial Rate of the Rat**

The non-selective β-adrenoceptor agonist, (±)isoproterenol, caused a concentration-dependent increase in the rate of contraction of the isolated rat right atria (Figure 1). The β2-adrenoceptor selective antagonist ICI 118 551 (1 μM) caused a small parallel rightward shift in the (±)isoproterenol concentration-response curve, altering the EC50 (effective concentration that produced 50% of the maximum effect) of (±)isoproterenol from 6.1 nM to 22 nM, a shift of 3.6-fold (Figure 1a). In contrast, the β1-adrenoceptor selective antagonist, CGP 20712A (1 μM), which has an affinity for β1-adrenoceptors similar to that which ICI 118 551 has for β2-adrenoceptors (Table 1), caused a larger rightward parallel shift in the (±)isoproterenol concentration-response curve, altering the EC50 of (±)isoproterenol from 3.9 nM to 570 nM, a 150-fold shift (Figure 1b). These results are consistent with a predominant role.
**CARDIOVASCULAR EFFECTS OF CLENBUTEROL**

**Table 1. Dissociation constants, selectivity, and receptor occupancy of \( \beta \)-adrenoceptor-selective antagonists**

<table>
<thead>
<tr>
<th>Item</th>
<th>ICI 118 551</th>
<th>CGP 20712A</th>
</tr>
</thead>
<tbody>
<tr>
<td>( K_d ) at ( \beta_1 ), nM ( ^a )</td>
<td>198</td>
<td>3.32</td>
</tr>
<tr>
<td>( K_d ) at ( \beta_2 ), nM ( ^a )</td>
<td>1.92</td>
<td>4910</td>
</tr>
<tr>
<td>Selectivity ( ^b )</td>
<td>102</td>
<td>1479</td>
</tr>
<tr>
<td>At .1 ( \mu M ) (antagonist) ( ^c )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% ( \beta_1 ) Occupied</td>
<td>33.6</td>
<td>96.8</td>
</tr>
<tr>
<td>% ( \beta_2 ) Occupied</td>
<td>98.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

\( ^a \) \( K_d \) values from Marullo et al. (1989).

\( ^b \) Selectivity is the potency of an antagonist for its high affinity receptor relative to its potency for its low affinity receptor (\( K_d \) of high-affinity receptor/\( K_d \) of low-affinity receptor).

\( ^c \) Percentage of occupancy determined from \( ([B]/[B]+K_d) \), where \([B]\) is the concentration of antagonist and \( K_d \) is the dissociation constant.

Figure 1. Concentration-response curves for (±)isoproterenol in spontaneously beating rat right atria. Each figure shows the curve produced in response to (±)isoproterenol and the response in the same tissues produced by (±)isoproterenol in the presence of \( \beta \)-adrenoceptor-selective antagonists. (a) \( EC_{50} \) values: (±)isoproterenol, 6.1 ± 1.2 nM; (±)isoproterenol + ICI 118 551, 22.4 ± 2.4 nM (b). (±)isoproterenol, 3.9 ± .5 nM; (±)isoproterenol + CGP 20712A, 568 ± 94 nM. Values are means ± SE (n = 4).

Figure 2. Concentration-response curves to CLEN showed that the \( \beta_2 \)-agonist was less potent than (±)isoproterenol, and had to be given at higher concentrations to increase heart rate (\( P < .001 \)). Furthermore, CLEN increased heart rate to only 45% of the maximum increase produced by (±)isoproterenol (Figure 2a), confirming that CLEN is only a partial agonist. This increase was completely abolished by \(.1 \mu M \) CGP 20712A (Figure 2b), consistent with its being a \( \beta_1 \)-adrenoceptor-mediated response.

Bovine Right Atrial Trabeculae

A significant increase in the force of contraction of the bovine right atrial trabeculae was caused by (±)isoproterenol (Figure 3). This effect was only slightly antagonized by ICI 118 551, which altered the \( EC_{50} \) for (±)isoproterenol from 51.7 nM to 223 nM, a 4.3-fold shift (Figure 3a). In contrast, the positive inotropic effect of (±)isoproterenol was significantly antagonized by CGP 20712A, which altered the \( EC_{50} \) for (±)isoproterenol from 23 nM to 8.6 \( \mu M \), approximately a 375-fold shift (Figure 3b). Again, these results are consistent with a predominant role for the \( \beta_1 \)-adrenoceptors in controlling force of contraction in bovine atria. As with rat data, pseudo \( pA_2 \) values can be calculated for the antagonists to determine the affinity of the antagonists for bovine \( \beta \)-adrenoceptor subtypes. Pseudo \( pA_2 \) values were 9.59 ± .04 and 7.61 ± .31 for CGP 20712A and ICI 118 551, respectively.

Bovine Infusion Studies

When administered intravenously, CLEN rapidly increased heart rate, which peaked and stabilized...
Figure 2. Concentration-response curves for (±)isoproterenol and clenbuterol in spontaneously beating rat right atria. Each figure shows the curve produced in response to (±)isoproterenol and the response in the same tissues produced by clenbuterol in the absence or presence of CGP 20712A. (a) EC50 values: (±)isoproterenol, 5.3 ± 1.6 nM; clenbuterol, 874 ± 264 nM. (b) (±)Isoproterenol, 8.3 ± 2.0 nM; clenbuterol + CGP 20712A did not produce a positive chronotropic response, and therefore no EC50 could be determined. Values are means ± SE (n = 4).

Figure 3. Concentration-response curves for (±)isoproterenol in bovine right atria. Each figure shows the curve produced in response to (±)isoproterenol and the response in the same tissues produced by (±)isoproterenol in the presence of β-adrenoceptor-selective antagonists. (a) EC50 values: (±)isoproterenol, 51.7 ± 29.2 nM; (±)isoproterenol + ICI 118 551, 223 ± 50 nM. (b) (±)Isoproterenol, 23.0 ± 6.1 nM; (±)isoproterenol + CGP 20712A, 8,600 ± 1,514 nM. It was a common observation in these studies that the basal force of contraction was reduced after (±)isoproterenol, used in the first concentration-response study. This phenomenon is not uncommon in inotropic studies. Values are means ± SE (n = 3).
Figure 4. An example of the effects of clenbuterol on heart rate and diastolic blood pressure in a Brahman-cross steer.

within 5 to 10 min. The increase in heart rate was accompanied by a fall in diastolic blood pressure that was compensated for within 10 to 15 min (Figure 4).

Infusion of 5 mg of CGP 20712A decreased heart rate by 30 bpm (or 54% of the increase produced by CLEN, Figures 4a, 5a), and a further 5 mg of CGP 20712A decreased heart rate by 1 bpm only. This indicated two things. First, approximately 50% of the increase in heart rate produced in response to CLEN was mediated by $\beta_2$-adrenoceptor stimulation. Second, 5 mg of CGP 20712A was sufficient to block $\beta_2$-adrenoceptor-mediated effects on the heart rate in cattle. Following the administration of CGP 20712A, the administration of ICI 118 551 completely antagonized the CLEN-stimulated increase in heart rate ($P < .01$), returning the heart rate back to near the control level. This indicated that the remaining effects of CLEN, which were not $\beta_2$-adrenoceptor mediated,
Further administration of IC1 118 551 (up to 5 mg), the control resting heart rate (Figures 4b, 5b). The affinity of IC1 118 551 for $\beta_2$-adrenoceptors is similar to that of CGP 20712A for $\beta_1$-adrenoceptors (Table 1). Because 5 mg of CGP 20712A blocked $\beta_1$-adrenoceptors, we chose to use 5 mg of IC 118 551 as the initial dose to block $\beta_2$-adrenoceptors. When 5 mg of IC 118 551 was administered, the antagonist reduced the CLEN-mediated increase in heart rate by 90% ($P < .05$; i.e., heart rate returned back to within 7 bpm of the control resting heart rate [Figures 4b, 5b]). Further administration of IC 118 551 (up to 20 mg) reduced the heart rate back to the control resting heart rate. This was accompanied by an apparent increase in diastolic blood pressure that just escaped statistical significance ($P = .12$; Figure 4b).

**Blood Parameters**

Clenbuterol caused an increase in the plasma glucose concentration ($P < .05$), which was slow in development relative to the increase in heart rate. This increase was not antagonized by either IC 118 551 or CGP 20712A over the time period investigated (Figures 6, 7a, 7b). Clenbuterol also caused a fall in the plasma potassium concentration ($P < .05$), which was similarly slow in development, and which also was not antagonized by either IC 118 551 or CGP 20712A over the time period investigated (Figures 6, 7c, 7d).

**Discussion**

**Role of $\beta$-Adrenoceptors in Rat and Bovine Right Atria**

The isolated, spontaneously beating rat right atrium is a preparation often used to determine the effects of positive chronotropic drugs such as $\beta$-agonists (Kaumann, 1986). The importance of the $\beta_1$- and $\beta_2$-adrenoceptors in positive chronotropy can be delineated by using subtype-selective antagonists, such as CGP 20712A and IC 118 551, that have similarly high affinities for their respective adrenoceptors (Table 1). These antagonists have been used to identify $\beta_1$- and $\beta_2$-adrenoceptor subtypes in a number of mammalian tissues, with similar $pA_2$ values being observed between different species. Most importantly, their high affinity for bovine $\beta_1$- and $\beta_2$-adrenoceptor subtypes has been demonstrated in skeletal muscle in studies of radioligand binding and adenylyl-cyclase activation (Silence and Matthews, 1994). In contrast to the rat, it is not possible to measure bovine heart rate in vitro to determine the predominant $\beta$-adrenoceptor subtype in the right atrium. This is due to the difficulty in isolating the large pacemaker intact (Kaumann and Marano, 1982). Instead, force of contraction (positive inotropic response) must be measured. Although this technique is well established for the human heart (Labow et al., 1991; Hoey et al., 1993), as far as we are aware this is the first time that it has been used for the bovine heart.

Using identical protocols in rat and bovine right atria, similar outcomes were obtained. The IC 118 551 caused a negligible rightward shift in the (±)isoproterenol $EC_{50}$, much smaller than that expected for a $\beta_2$-adrenoceptor mediated response. A shift of this magnitude can be explained by partial blockade of functional $\beta_1$-adrenoceptors: the selectivity of IC 118 551 is such that 33% of the $\beta_1$-adrenoceptors are occupied at the concentration of drug required to saturate the $\beta_2$-adrenoceptors (Table 1). Thus, this small shift in $EC_{50}$ indicates that the $\beta_2$-adrenoceptor population must play only a minor role, if any, in the positive chronotropic and inotropic effects of $\beta$-agonists in rat and bovine right atria, respectively. If used at an appropriate concentration, CGP 20712A can saturate $\beta_1$-adrenoceptors without occupying a significant number of $\beta_2$-adrenoceptors (Table 1). Thus, in the presence of a non-selective agonist such as isoproterenol, the $EC_{50}$ for a $\beta_1$-adrenoceptor-mediated response is shifted markedly to the right, whereas the $EC_{50}$ for the $\beta_2$-adrenoceptor mediated component is unaltered. The presence of both sub-types results in a distinctly biphasic agonist-response curve, as observed in rat atria by Kaumann (1986). However, in the present study, the response to isoproterenol in the presence of CGP 20712A was clearly monophasic in both species. Furthermore, the large shift in the $EC_{50}$ for (±)isoproterenol is consistent with blockade of the $\beta_1$-adrenoceptor population. Thus, it seems that the predominant functional $\beta$-adrenoceptor subtype in rat and bovine right atria is the $\beta_1$-adrenoceptor subtype.

**Rationale for Dose of Clenbuterol**

Clenbuterol and the $\beta$-adrenoceptor antagonists were administered to steers intravenously to induce a rapid response as well as to avoid loss of the drug during absorption from the gastrointestinal tract. The dose of CLEN used in the present study (up to 1 mg) was designed to increase heart rate by approximately 50 bpm, which is submaximal. This prevented undue stress to the animal and the precipitation of side effects such as muscle tremor, but was sufficient to allow us to measure antagonist-induced changes. In addition to increasing heart rate, the dose of CLEN caused other responses seen in previous experiments when CLEN was administered as a repartitioning agent. Thus, plasma glucose concentrations were increased similarly to those reported previously when CLEN was fed to cattle (Eisemann et al., 1988; Bruckmaier and Blum, 1992). Furthermore, this dose of CLEN caused a significant fall in plasma potassium concentrations, which is a characteristic $\beta_2$-adrenoceptor-mediated response (Brown, 1985). These metabolic effects were slower in onset than the cardiovascular changes, possibly due to the more complex series of
Figure 5. The effects of clenbuterol and β-adrenoceptor selective antagonists on heart rate in vivo. For each steer an average value was calculated from four heart rate readings taken 5 min apart before addition of the next drug. Each column represents the mean ± SE of such values obtained in four steers.

Figure 6. An example of the effects of clenbuterol on plasma glucose and potassium concentrations in a Brahman-cross steer.
biochemical reactions that are required for these effects to be seen. The slower kinetics of onset may suggest why these metabolic effects were not reversed in the short time frame examined, following the administration of a CLEN antagonist. Nevertheless, the changes in plasma glucose and potassium concentrations are indicators that at the intravenous dose administered, CLEN was producing effects at the tissue level similar to those seen when the drug is added to the diet. Hence, this intravenous dose was appropriate to examine the effects of CLEN on the cardiovascular system.

Mechanisms Through Which Clenbuterol Increases Heart Rate

There are three different possible mechanisms through which CLEN could increase heart rate. The first possibility is that the effects of CLEN are directly β₁-adrenoceptor mediated (Figure 8). This possibility is supported by the findings that CLEN is not highly selective for the β₂-adrenoceptor (Cohen et al., 1982) and that the predominant β-adrenoceptor subtype in the rat and bovine right atria is the β₁-adrenoceptor. Furthermore, the positive chronotropic effects of CLEN were abolished in rat atria by a β₁-antagonist. However, there are two findings that lead us to eliminate this possibility. First, from the data in vitro, CLEN is only a partial agonist at the rat β₁-adrenoceptor. If CLEN is also a partial agonist at the bovine β₁-adrenoceptor, then by definition it must occupy 100% of the β₁-adrenoceptors to produce its own maximum response. Thus, to elevate heart rate even submaximally, CLEN would have to be present in the blood at very high levels, possibly even millimolar concentrations, whereas the highest plasma concentrations ever reported in cattle are equivalent to only 5.4 nM, after 21 d of administration (Meyer and Rinke, 1991). More direct evidence against this mechanism was obtained from our cattle studies in vivo. If CLEN had produced its positive chronotropic response through direct β₁-adrenoceptor
Figure 8. Possible mechanisms of action through which clenbuterol increases heart rate. (1) Clenbuterol stimulates cardiac $\beta_1$-adrenoceptors, an effect that can be antagonized by CGP 20712A but not by ICI 118 551. (2) Clenbuterol stimulates cardiac $\beta_2$-adrenoceptors, an effect that can be blocked by ICI 118 551 but not by CGP 20712A. (3) Clenbuterol stimulates vascular $\beta_2$-adrenoceptors to lower blood pressure and stimulates the baroreceptor reflex, an effect that can be totally blocked by ICI 118 551 and partially blocked by CGP 20712A according to the extent to which this reflex is mediated by sympathetic nervous system activity at $\beta_1$-adrenoceptors.

stimulation, this effect would have been resistant to blockade by ICI 118 551. This is not what we observed; the tachycardiac response to CLEN was eliminated completely by ICI 118 551. Thus, it is extremely unlikely that CLEN produces its positive chronotropic effect in vivo through direct stimulation of the cardiac $\beta_1$-adrenoceptors.

The second possible mechanism of action is that CLEN directly stimulates $\beta_2$-adrenoceptors in the heart (Figure 8). A minor population of functional $\beta_2$-adrenoceptors has been reported to exist in rat atria (Kaumann, 1986), although the present data do not support this, neither do they support that the effect of CLEN in isolated rat atria is $\beta_2$-adrenoceptor-mediated. It would have been useful to be able to isolate and characterize the bovine pacemaker (sinoatrial node). However, using the closest system we could (isolated bovine atrial tissue), again we found no compelling evidence for functional $\beta_2$-adrenoceptors. Our observations in vivo showed that 50% of the response to CLEN was eliminated by the $\beta_1$-selective antagonist, CGP 20712A. Thus, at least half of the chronotropic response could not have been mediated by stimulation of cardiac $\beta_2$-adrenoceptors. Although we cannot rule out the possibility that cardiac $\beta_2$-adrenoceptors localized in the sinoatrial node contributed to the remaining 50%, our data suggest this to be unlikely, and instead support a third proposed mechanism of action.

The third possible mechanism is that CLEN stimulates $\beta_2$-adrenoceptors on vascular smooth muscle to produce vasodilation (Cohen et al., 1982) and hypotension, resulting in the activation of pressure-sensitive receptors. These receptors are known as baroreceptors, and their activation leads to a reflex increase in heart rate via several mechanisms. This reflex is known as the baroreceptor reflex (Guyton, 1986).

The mechanisms through which CLEN produces its effects in cattle via the baroreceptor reflex have been elucidated in this study. The CGP 20712A reduces the CLEN-mediated increased heart rate by approximately 50%, showing a major role for the $\beta_1$-adrenoceptors in the actions of the baroreceptor reflex. The cardiac $\beta_1$-adrenoceptors could be stimulated directly by circulating or local catecholamines. This proposal is supported by previous findings that CLEN causes an increase in plasma norepinephrine levels, with little change in plasma epinephrine or dopamine levels (Mersmann, 1989). This norepinephrine may be released from the adrenal gland or could emanate from sympathetic nervous system overflow. Stimulation of the vasoconstrictor center of the medulla, via the baroreceptor reflex, will cause such a release of norepinephrine (Guyton, 1986). Norepinephrine acts in a homeostatic manner to increase the blood pressure toward normal. Norepinephrine is a full agonist selective for $\beta_1$-adrenoceptors and stimulates cardiac $\beta_1$-adrenoceptors directly to increase the heart rate and blood pressure and stimulates $\alpha$-adrenoceptors to produce vasoconstriction to elevate blood pressure.

The remaining elevation in heart rate, which is CGP 20712A-resistant, could be due to two possibilities. First, as discussed above, direct $\beta_2$-adrenoceptor stimulation in the heart by CLEN would increase heart rate, although our studies in vitro provided no evidence for this. The second more probable mechanism is a decline in the cholinergic input from the
medulla, mediated via the baroreceptor reflex. The activation of the baroreceptor reflex also inhibits the vagal center to increase heart rate and force of contraction (Guyton, 1986). The increase in heart rate can only be reversed by blocking the cholinergic nerves, or by reversing the original cause of the vasodilation, such as with ICI 118 551.

The results of the present study allow a new interpretation of previously published work. Clenbuterol increases heart rate for the first few days of administration; however, heart rate then falls to near normal levels (Williams et al., 1986) or to levels elevated by only 50% of that seen after the initial dose of CLEN (Eisemann et al., 1988). After 14 d of CLEN administration, Bruckmaier and Blum (1992) were unable to elevate heart rate with CLEN, and yet heart rate could still be elevated with isoproterenol or exercise, which stimulates the sympathetic nervous system. The response to isoproterenol or exercise is consistent with the stimulation of cardiac β1-adrenoceptors, which are unlikely to have downregulated greatly following the administration of the β2-adrenoceptor agonist CLEN. However, the reason why heart rate could not be elevated with CLEN was probably due to adaptation of the baroreceptor reflex. It is well documented that persistent hypotension will result in adaptation of the baroreceptors, such that after a couple of days the input to the medulla from the baroreceptors returns to normal (Guyton, 1986). This returns the heart rate to nearly normal levels and prevents continuous hypotension from resulting in a continuously elevated heart rate.

There are several implications from this work. It is important to note that CLEN produced significant cardiovascular effects, but that these effects decrease after a few days. It is interesting that when the increased heart rate and blood flow effects subside (Eisemann et al., 1988), the increased energy expenditure subsides similarly (MacRae et al., 1988), suggesting that they are linked. These cardiovascular stresses and increased sympathetic nervous system activity may also be a cause for the appetite suppression initially seen after the administration of CLEN. Because the cardiovascular effects of CLEN are due to β2-adrenoceptor-mediated vasodilation, such effects could not be eliminated by any modification of CLEN, without also reducing its muscle repartitioning effects, which are also β2-adrenoceptor-mediated. Furthermore, these effects are likely to occur with all β2-agonists used as muscle repartitioning agents.

Nevertheless, the cardiovascular effects of CLEN can be significantly reduced by the concurrent administration of a highly selective β1-adrenoceptor antagonist. Such a drug may reduce heart rate by 50%, possibly without affecting the muscle repartitioning effects of CLEN. After the first few days of this drug combination, the baroreceptors should be sufficiently adapted to allow withdrawal of the antagonist and continued administration of CLEN alone. This combined treatment regimen may also help reduce the transient increase in energy expenditure and loss of appetite seen following the initial administration of CLEN.

Implications

Clenbuterol is a powerful repartitioning agent but has the adverse effect of increasing heart rate markedly for the first few days of administration. This may cause an increase in energy expenditure and loss of appetite. We found the increase in heart rate to be a reflex response caused by a fall in blood pressure. The fall in blood pressure is mediated by β2-adrenoceptors, which also increase muscle growth and so cannot be avoided with this type of drug. However, the reflex increase in heart rate also involves other receptors and can be reduced by approximately 50% by a second drug that is unlikely to counteract the beneficial actions of clenbuterol.

Literature Cited


