

Human Risk Assessment of Beta Adrenoceptor Agonists Use in Food-Producing Animals

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INTRODUCTION

Beta-adrenoceptor agonists (BAAs) mimic the actions of adrenaline and noradrenaline, mainly, but not exclusively, at various Beta-adrenoceptor sites. Many BAAs were developed as cardiac stimulants or as bronchodilators for clinical use. While some of these drugs and other new BAAs have been investigated in food-producing animals for their ability to reduce body fat content, increase muscle mass and improve growth rate, none has yet been approved for such application in Canada. Recently, illegal use of clenbuterol, a bronchodilator, as a repartitioning agent in young cattle and associated human food poisonings by residues of this BAA in veal liver were reported in several countries.

The health risk of BAAs residues was assessed based on their mechanism of action, toxicity, pharmacokinetic profiles and clinical experience in humans. It was concluded that selectivity for various sub-types of Beta-adrenoceptors is not absolute and there is potential for residues of BAAs to activate cardiac Beta₁-adrenoceptors in humans.

MECHANISM OF ACTION OF BETA ADRENOCEPTOR AGONISTS

Table I. Drugs that act on beta adrenoceptors and their location.

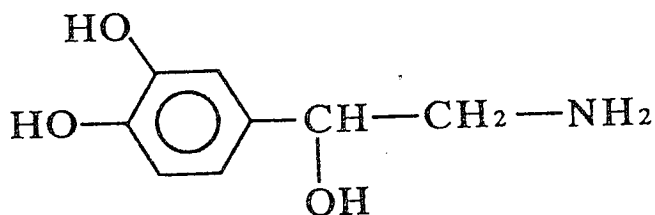
RECEPTOR	AGONISTS	ANTAGONISTS	TISSUE	RESPONSES
BETA ₁	ISO > AD = NAD DOBUTAMINE	METOPROLOL	HEART	Increased force and rate of contraction and nodal conduction velocity.
			JG CELLS	Increased renin secretion.
BETA ₂	ISO > AD >> NAD TERBUTALINE	ICI 118551	SMOOTH MUSCLE	Relaxation
			SKELETAL MUSCLE	Glycogenolysis, uptake of K ⁺
			LIVER	Glycogenolysis, gluconeogenesis
BETA ₃	ISO = NAD > AD BRL 37344	ICI 118551 CGP 20712A	ADIPOSE TISSUE	Lipolysis

Adrenaline (AD), Noradrenaline (NAD), Isoprenaline (ISO), Juxtaglomerular cells (JG).
Modified from Goodman and Gilman's - The Pharmacological Basis of Therapeutics, Eighth Edition, 1990.

Table II. Structure activity relationship. Effect of n-substitution on the adrenoceptor stimulant activity of noradrenaline. Addition of a methyl group to the terminal nitrogen atom (adrenaline) conveys considerable beta-adrenoceptor stimulant potency whilst addition of an isopropyl group (isoprenaline) further increases beta-, but practically removes alpha-adrenoceptor stimulant activity.

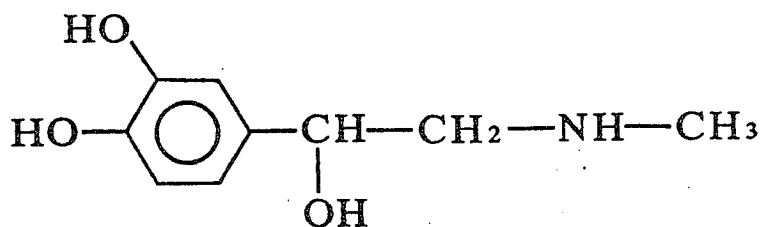
SYMPATHOMIMETIC SUBSTANCE

ACTIVITY



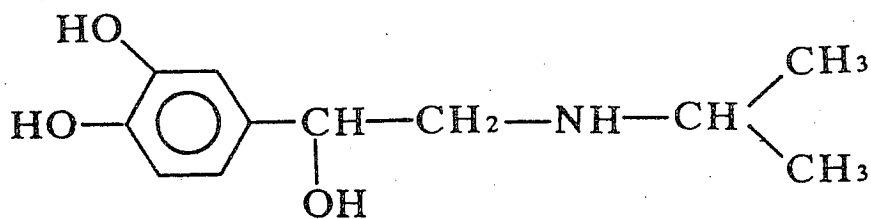
NORADRENALINE

MAINLY ALPHA
BUT STRONG BETA₁
EFFECT ON THE
HEART.



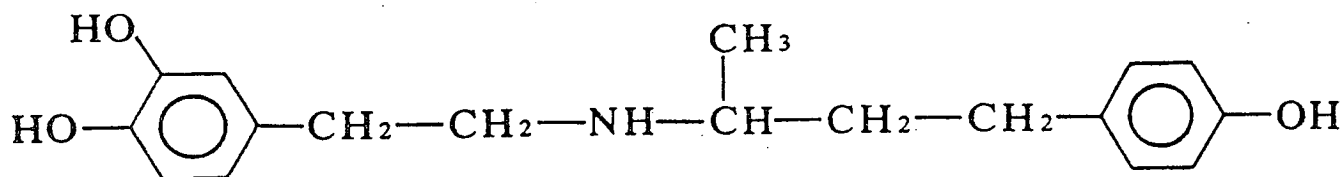
ADRENALINE (N-METHYLNORADRENALINE)

ALPHA & BETA

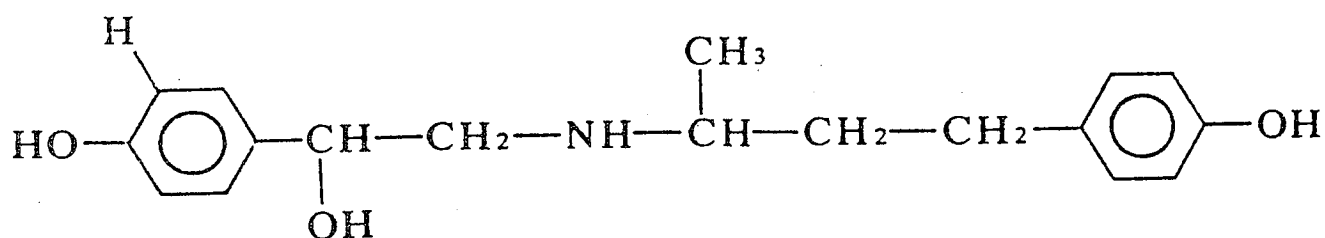


ISOPRENALINE (N-ISOPROPYLNORADRENALINE)

MAINLY BETA



DOBUTAMINE

MAINLY BETA₁

BUTOPAMINE

MAINLY BETA₁

Table III. Selectivity for rat brown adipocyte lipolysis over atria and trachea.

AGONIST	ATRIA	TRACHEA
Isoprenaline	0.14	0.23
Fenoterol	0.21	0.02
Salbutamol	0.35	0.01
BRL 37344	400	20.5

Selectivity ratios are ratios of EC₅₀ values (table modified from Arch et al. 1984).

MECHANISMS OF ACTION FOR THE ANABOLIC EFFECT AND REDUCTION OF FAT OF SYMPATHOMIMETIC AGENTS

Nutting (1982) showed that adrenaline stimulates alpha-aminoisobutyric acid (AIB) uptake and incorporation of leucine and tyrosine into protein by isolated diaphragms from hypophysectomised rats. Li and Jafferson (1977) observed that isoprenaline increases alanine

utilisation and causes formation of glutamine instead of alanine. Emery *et al.* (1984) showed that clenbuterol increases the protein content and mitochondrial guanosine diphosphate (GDP) - binding capacity of brown adipose tissue. These workers further observed that clenbuterol does not affect plasma insulin, growth hormone, or triiodothyronine levels. However, fenoterol, reduces insulin levels. Body composition analysis reveals that clenbuterol does not affect body fat content but produces an increase in fat-free mass by raising water and protein content. From the literature cited, one can conclude that the mechanism of action for the anabolic effect and reduction of fat of sympathomimetic agents is currently unknown.

CARCINOGENICITY

BAA's induce mesovarian leiomyomas in Sprague-Dawley rats (Nelson and Kelly, 1971; Nelson *et al.* 1972). This effect is blocked by propranolol, a non-selective beta-adrenoceptor antagonist (Jack *et al.* 1983). Some of the BAA's that cause these tumours and their effective doses are shown in Table IV.

Table IV. Some of the beta adrenoceptor agonists that induce mesovaria leiomyomas in sprague-dawley rats.

<u>BAA</u> <u>2-YEAR STUDY</u>	<u>EFFECTIVE DOSE</u> <u>MG/KG/DAY</u>
Salbutamol	20
Terbutaline	6, 12, 60
Mesuprine	40, 100, 250
Soterenol	4.6, 10, 21.5

To summarize, a variety of chemical types of BAA's have been shown to induce mesovarian leiomyomas in Sprague-Dawley rats and their relative activities in this respect are compatible with their known activities at beta₂ receptors and their bioavailabilities in the rat (Jack *et al.* 1983).

PHARMACOKINETICS

The pharmacokinetics of clenbuterol in humans is discussed because the drug is the most studied BAA and the one whose residues in food-producing animals have been associated with human poisoning. Furthermore, data on the pharmacokinetics of BAA's in

animals are not available. The dichlorination of the aromatic ring in positions 3 and 5 enhances the lipid solubility of clenbuterol and results in rapid absorption following oral administration. Clenbuterol has a large volume of distribution and a long duration of action which are due, respectively, to its high lipid solubility and the fact that it is neither sensitive to the action of catechol-O-methyl transferase (COMT) nor to that of monoamine oxidase (MAO). Some biotransformation of the drug is believed to occur but its nature and extent are not known. The drug is eliminated from the body mainly in the urine, slowly, by glomerular filtration of the unchanged parent compound. The elimination half-life of clenbuterol in normal human volunteers is about 34 hours (Tschan et al. 1979; Menard, 1984).

CLENBUTEROL RESIDUES IN VEAL LIVER ASSOCIATED WITH AN OUTBREAK OF FOOD POISONING IN HUMANS

Twenty-two people complained of the signs and symptoms shown in Table V, 1-3 hours after eating veal liver. Assays of clenbuterol in samples of veal showed concentrations of 0.375 and 0.500 mcg/g in two samples (Pulce et al. 1991). Clenbuterol was assayed by gas chromatography-negative-ion chemical ionisation mass spectrometry with methane as the reagent gas. This method has a detection limit of 5 pg ml⁻¹ for plasma samples (Cirault and Fourtillan, 1990).

Table V. Signs and symptoms of clenbuterol poisoning in humans.

Headache
Tremors
Palpitations
Dizziness
Tachycardia
Malaise

DISCUSSION

When fed to growing animals, several BAAs increased muscle and decreased fat mass (Emery et al. 1984; Fiems 1987; Ricks et al. 1984). However, the mode of action of BAAs in producing these effects is unknown and no member of this class of compounds has yet been approved for such application in food-producing animals in Canada. Recently, illegal use of clenbuterol, a bronchodilator, as a repartitioning agent in young cattle and associated human food poisonings by residues of this BAA were reported in several countries (Pulce et al. 1991).

Some of the signs and symptoms of clenbuterol poisoning (Table V) are identical to the side-effects of dobutamine a racemic and selective beta₁-adrenoceptor agonist

(Sonnenblick *et al.* 1979; Tuttle and Mills, 1975; Ruffolo and Yaden, 1983). Tachycardia, which is a prominent finding in cases of clenbuterol poisoning is presumably due to both a direct effect of this drug (O'Donnell, 1976) and a reflex action in response to hypotension caused by vasodilatation.

Arch *et al.* (1984) studied the selectivity of some BAAs for brown adipocyte lipolysis over atria and trachea in rats (Table IV). From this table it is observed that the EC_{50} ratio of the selective β_3 -adrenoceptor agonist, BRL 37344, for brown adipocyte lipolysis over atria and trachea is 400 and 25 respectively. These very high figures for BRL 37344, indicate that during ordinary use, this compound will only have an effect at the atrial β_1 - and the tracheal β_2 -adrenoceptors at doses that are 400 X, and 25 X higher, respectively, than those that cause brown adipocyte lipolysis. BRL 37344, thus provides the ultimate in selectivity, for compounds that affect brown adipocyte lipolysis, while other BAAs studied and shown in Table IV, are relatively devoid of such selectivity.

The method by Arch *et al.* (1984) of evaluating the selectivity of the BAAs is a very attractive one. However, this method cannot be widely advocated for all potential repartitioning agents in food-producing animals because the relationship between brown adipocyte lipolysis and the reduction in fat mass is not established (Fiems, 1987). Taken together, the studies of O'Donnell (1976), Arch *et al.* (1984) and the signs and symptoms of clenbuterol poisoning (Pulce *et al.* 1991) show that selectivity for various sub-types of beta-adrenoceptors is not absolute and there is potential for residues of BAAs to activate cardiac β_1 -adrenoceptors in humans.

Salorine *et al.* (1975) observed that clenbuterol 10 mcg three times a day orally, was effective in relieving asthma. Dobutamine at 2.5 to 10 mcg/kg/min, intravenously, was found effective in increasing myocardial contractility and cardiac output in patients with cardiac failure (Sonnenblick *et al.* 1979). Butopamine at 0.04 to 0.08 mcg/kg/min, intravenously, caused substantial positive inotropy. Positive chronotropy was observed at >0.08 mcg/kg/min (Nelson and Leier, 1981). These findings support the hypothesis that BAAs are extremely potent drugs, particularly in humans. Since the BAAs are effective at such low doses, highly sensitive analytical methods, with parts per trillion analytical capabilities, should be available to monitor for their residues in edible tissues of food-producing animals (Cirault and Fourtillan, 1990).

Table IV shows some of the BAAs that cause mesovarian leiomyomas in Sprague-Dawley rats (Nelson and Kelly, 1971; Nelson *et al.* 1972). This effect is blocked by propranolol a non-selective beta-adrenoceptor antagonist (Jack *et al.* 1983). The mesovarian smooth muscle cells contain beta-adrenoceptors which mediate cellular relaxation. The proliferation of mesovarian muscle caused by beta-stimulants in the rat may be an attempted physiological adaptation to continuous relaxation of the muscle.

A variety of chemical types of beta-stimulants have now been shown to induce leiomyomas and their relative activities in this respect are compatible with their known activities at β_2 receptors and their bioavailabilities in the rat: they include sulphonanilides (soteranol, mesuprine and zinterol), resorcinols (terbutaline, reproterol) and a saligenin (salbutamol). Accordingly, it is now virtually certain that the negative data reported with other beta stimulants result from failure to take proper account of their absolute potencies

and bioavailabilities in the rat and/or the use of relatively insensitive strains of rats in the experiments (Jack et al. 1983).

To date, there is no evidence to associate the BAAs, such as salbutamol, with tumour induction in humans (Poynter et al. 1978). Some beta blockers, for example pronethalol, are carcinogenic in mice (Alcock and Bond 1964). However, it was concluded that any carcinogenic potential of these compounds was compound specific and not due to their beta blocking activity (Jackson and Fishbein, 1986).

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