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The Adrenergic Control of Lower Esophageal Sphincter Function: Response to Beta$_2$ Adrenergic Agonists (38730)

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(Introduced by F. P. Brooks)

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Lands and co-workers have demonstrated a significant degree of selectivity of action at the beta adrenergic receptors of different tissues (1–3). Two types of beta adrenergic receptors, beta$_1$ and beta$_2$, have been proposed. Beta adrenergic effects on the heart are mediated by beta$_1$ receptors while the effects on bronchial and vascular smooth muscle are mediated by beta$_2$ receptors (4–6). Although several selective beta$_2$ adrenergic agonists have been synthesized, there is little information on the nature of the beta adrenergic receptors of the gastrointestinal tract. We have recently demonstrated that the lower esophageal sphincter (LES) of the opossum, an animal with a smooth-muscle distal esophagus similar to man, showed a dose-related inhibitory response to beta adrenergic stimulation (7). The purpose of this study was to compare the LES inhibitory response of isoproterenol, a beta adrenergic agonist with effects at both types of beta receptors, with the selective beta$_2$ adrenergic agonists, salbutamol and carbuterol. Our aim is to determine whether a beta$_2$ adrenergic agonist can be used to reduce basal LES pressure without significantly altering the blood pressure or heart rate.

Methods. All studies were performed on the adult opossum, species *Didelphis virginiana*. Male and female animals weighing between 2.2 and 4.6 kg were anesthetized with 2.0% chloralose (3.0 cm^3/kg). After successful anesthesia, a heparinized cannula was inserted into the femoral artery and connected to an external transducer (Statham Instruments, Inc., Oxnard, CA) for constant blood pressure monitoring. A similar cannula was inserted into the opposite femoral vein for drug administration. Patency of this cannula was maintained through a slow infusion of 0.9% saline.

Intraluminal pressures were measured through water-filled polyvinyl catheters, 1.4 mm i.d., connected to external transducers (Statham P23BB) with a linear external calibration of 0–250 mmHg. Recording tubes were arranged as a fixed unit with three side orifices, 1.2 mm in diameter, spaced 5 cm apart over the distal segment of the tube. Each recording tube was constantly perfused with distilled water by a Harvard infusion pump (Harvard Apparatus Co., Inc. Millis, MA) at 1.2 cm^3/min. After insertion into the animal's stomach, the entire recording assembly was withdrawn at 0.5-cm intervals with measurements being recorded at each level for 1 min. For changes in LES pressure in response to drugs, the middle recording orifice was positioned in the sphincter with the proximal lumen in the esophagus and the distal pressure orifice in the stomach. The middle orifice was maintained at the zone of maximal LES pressure by the evaluation of a complete pull-through at each 5-min interval. To evacuate the stomach, gastric aspiration was done through the distal orifice approximately every 30 min. All intraluminal pressures, as well as blood pressure, were graphed on a Beckman rectilinear, ink-writing recorder (Beckman Instruments, Inc., Fullerton, CA).

LES pressure was recorded as millimeters of mercury with gastric fundal pressure used as a zero reference. The values were obtained as the midrespiratory value during a 1-min interval from the portion of the sphincter demonstrating the highest pressure. Mean LES pressure was obtained by determining the stable pressure at a minimum of four points during a 1-min period. Blood pressure was measured as the mean pressure obtained over a 1-min interval. Swallowing was induced by gently touching the cricoid cartilage.

Control values of LES pressure, percentage of relaxation of the LES with swallowing, and blood pressure were obtained after insertion of the intravenous and intra-arterial cannulas and before the instillation of drugs. A period of 30 min for stabilization to baseline levels was observed after the administration of each pharmacological agent.

The following pharmacological agents were given intravenously as 30-sec boluses: isoproterenol, salbutamol, and carbuterol. The dose range was 0.001 to 20.0 μg/kg. Drugs were given in random order and at random doses. Carbuterol and salbutamol were diluted in normal saline and administered as a 2.0 cm^3 injection. Isoproterenol was diluted in 5% dextrose and water and given at the same volume.

Statistical analysis was performed using the Student t test.

Results. In 23 animals, intraluminal manometry demonstrated the presence of a 1.6 $\pm$ 0.2 cm (mean $\pm$ SEM) zone of elevated pressure at the gastroesophageal junction. The mean midrespiratory pressure was 32.7 $\pm$ 5.6 mmHg above the intra-abdominal pressure.

In Figs. 1, 2, and 3, are shown the dose-response curves for (1) percentage inhibition in LES pressure, (2) percentage inhibition in mean arterial blood pressure, and (3) increase in heart rate. The LES pressure showed a similar maximal inhibition with each agonist. Isoproterenol was the most potent and carbuterol was the least potent. The ED_{50} (equivalent dose required to reduce LES pressure by 50% of the maximal observed inhibition) for isoproterenol was 0.075 μg/kg, while the ED_{50} values for salbutamol and carbuterol were 0.35 μg/kg and 1.0 μg/kg, respectively.

In contradistinction to LES pressure, blood pressure (Fig. 2) could be reduced by only 16.8 $\pm$ 1.6% with carbuterol as compared with 35.1 $\pm$ 5.0% with isoproterenol and 37.8 $\pm$ 7.7% with salbutamol. The percentage reduction in blood pressure was significantly less with carbuterol than with either isoproterenol ($P < 0.01$) or salbutamol ($P < 0.01$). The responses to isoproterenol and salbutamol were similar ($P > 0.05$). The ED_{50} for isoproterenol was 0.04 as compared to 0.30 for salbutamol and 0.35 for carbuterol.

The dose-response curves for increase in apical heart rate (Fig. 3), again, differed for each agonist. The observed maximal increase in heart rate with carbuterol (40.0 $\pm$ 6.9 beats/min) differed significantly

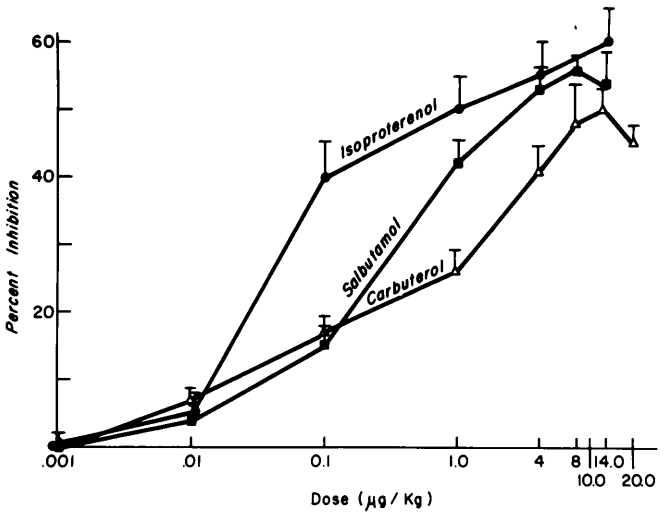

FIG. 1. Change in lower esophageal sphincter (LES) pressure in response to the intravenous administration of isoproterenol, salbutamol, and carbuterol. Each point represents the mean + SEM for the percentage inhibition in LES pressure, in studies performed in a minimum of eight animals.

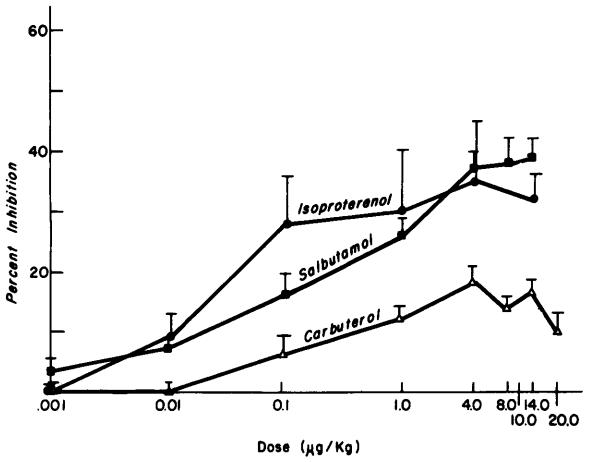

FIG. 2. Change in blood pressure in response to the intravenous administration of isoproterenol, salbutamol, and carbuterol. Each point represents the mean + SEM for the percentage inhibition in mean arterial blood pressure in studies performed in a minimum of eight animals.

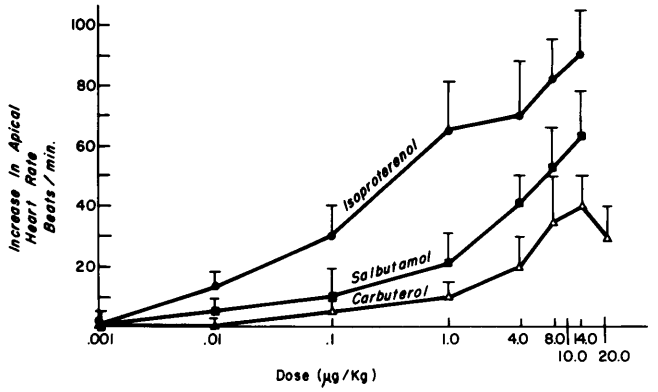

FIG. 3. Change in heart rate in response to the intravenous administration of isoproterenol, salbutamol, and carbuterol. Each point represents the mean + SEM for the absolute increase in heart rate in beats/minute in studies performed in a minimum of eight animals.

($P < 0.05$) from that observed with either isoproterenol (90.2 ± 14.4 beats/min) or salbutamol (63.0 ± 14.8 beats/min). The observed maximal increase in heart rate was not statistically different for isoproterenol as compared to salbutamol. The respective ED_{50} for each agonist was 0.25 µg/kg for isoproterenol, 2.0 µg/kg for salbutamol, and 4.0 µg/kg for carbuterol. These data indicate that: (1) isoproterenol is the more potent agonist for each of the three parameters measured, carbuterol the least potent; (2) the maximal response to each agonist was similar for percentage inhibition in LES pressure, but carbuterol gave a significantly smaller change in blood pressure and heart rate.

In Table I is shown a comparison of the blood pressure and heart rate effects for each agonist at the ED_{50} for LES inhibition. At the ED_{50} for isoproterenol the effects on blood pressure and heart rate were greater than for carbuterol. The effect of salbutamol on heart rate but not blood pressure was less than isoproterenol at the ED_{50} for percentage inhibition in LES pressure. The maximal response to each agonist could be antagonized to less than 10% of its control value using propranolol (0.2 mg/kg).

Discussion. The purpose of this study was

TABLE I. COMPARATIVE CHANGES IN CARDIOVASCULAR RESPONSE AT THE RESPECTIVE ED_{50} FOR LES PRESSURE REDUCTION IN RESPONSE TO EACH ADRENERGIC AGONIST.

	ED_{50} for percent inhibition in LES pressure (μg/kg)	Percent decrease in blood pressure	Increase in heart rate (beats/min)
Isoproterenol	0.075	23.0%	26.0
Salbutamol	0.35	21.0%	15.5
Carbuterol	1.0	12.5%	10.0

to further characterize the beta adrenergic response of the LES of the opossum by the use of selective beta$_2$ adrenergic agonists. The results of these studies suggested: First, selective beta$_2$ adrenergic agonists were less potent than isoproterenol in reducing LES pressure, but both carbuterol and salbutamol gave a similar maximum decrease in LES pressure; second, carbuterol acted as a partial agonist in reducing blood pressure and increasing heart rate as compared to isoproterenol and salbutamol. The separation in LES inhibition and cardiovascular responses with carbuterol but not with isoproterenol is evidence that the LES smooth muscle responds similarly to tracheobronchial smooth muscle, the suggested prototype of a structure containing the beta$_2$ subtype adrenergic receptor.

Lands and co-workers demonstrated that a significant degree of selectivity of action at beta adrenergic receptors in various tissues could be achieved by chemical alterations in the phenylethylamine moiety of catecholamines (1–3). Two types of beta adrenergic receptors, beta$_1$ and beta$_2$, were proposed to explain the differential responses of the heart as compared to the bronchial and vascular smooth muscle. Salbutamol and carbuterol have been shown to have a more selective effect at the proposed beta$_2$ receptor of the tracheobronchial tree than at the beta$_1$ receptor of the heart (5, 6, 8). This conclusion was based upon the observation that carbuterol and, to a lesser extent, salbutamol were less potent than isoproterenol in reducing blood pressure and increasing heart rate at a dose range shown to give a reduction in airway resistance.

The studies described in this report indicated that LES pressure can be reduced with selective beta$_2$ adrenergic agonists and this reduction in pressure was of a similar magnitude as that achieved with isoproterenol, the adrenergic agonist acting at both the beta$_1$ and beta$_2$ receptor. This observation suggested that the LES smooth muscle responded similarly to tracheobronchial smooth muscle rather than cardiac muscle. Whether this differential response of the gastrointestinal and cardiovascular systems to carbuterol indicated two specific types of beta adrenergic receptors cannot be proved from these data. We support the hypothesis of Lands and co-workers, and suggest that the LES of the opossum may contain a beta$_2$ adrenergic receptor. Further studies on the actual tissue-receptor mechanism are needed to confirm specific differences in types of beta adrenergic receptors. The possibility of a single receptor type with different affinities for specific agonists has not been excluded by studies reported to date.

The description of an adrenergic agonist capable of reducing LES pressure with minimal change in blood pressure and heart rate may prove useful in the pharmacological management of achalasia. Presently, significant clinical reduction in LES pressure can be achieved only with surgery or forceful disruption of the sphincter. It is possible that a beta$_2$ adrenergic agonist may be useful in providing prolonged periods of reduced LES pressure. If human studies do show encouraging results in lowering LES pressure, a controlled trial is indicated prior to clinical recommendation of the beta$_2$ adrenergic agonists for achalasia or diffuse esophageal spasm.

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