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INVESTIGATION OF OCCURRENCE OF TOLERANCE TO BRONCHODILATOR DRUGS IN CHRONICALLY PRETREATED GUINEA-PIGS

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1 The actions of sympathomimetic amines on isolated preparations of atria, trachea and ileum were studied *in vitro* in guinea-pigs, which had been pretreated for 5 or 12 days, by subcutaneous injection, with adrenaline (5 μg/kg), salbutamol (0.5 μg/kg), methoxamine (250 μg/kg) or saline (0.9% w/v NaCl solution).

2 In the trachea, a decrease in sensitivity (tolerance) to the relaxant effect of adrenaline was induced by pretreatment, for 12 but not for 5 days, with adrenaline. In these animals, cross-tolerance to isoprenaline or salbutamol was not observed. Tolerance to the relaxant actions of adrenaline, isoprenaline or salbutamol was not observed after pretreatment with salbutamol.

3 In the trachea, pretreatment with methoxamine or adrenaline for 12 days did not change the sensitivity to the α-adrenoceptor-mediated contractor action of methoxamine.

4 In the atria from those guinea-pigs pretreated with adrenaline or salbutamol, there was no reduced sensitivity to the β-adrenoceptor agonist actions of adrenaline, isoprenaline or salbutamol. In animals pretreated with methoxamine or adrenaline, there was no observable tolerance or cross tolerance to methoxamine with respect to its α-adrenoceptor-mediated positive inotropic action in the atria and no unequivocal evidence of a reduced sensitivity to that action of adrenaline.

5 It was confirmed that the twitch-like contractions of the longitudinal muscle of the electrically stimulated ileum were inhibited by sympathomimetic amines acting on α- and β-adrenoceptors. There was no reduced sensitivity to the inhibitory actions of noradrenaline or isoprenaline on the twitch of ileum isolated from animals pretreated with adrenaline, salbutamol or methoxamine for 5 or 12 days.

6 From our results on these three preparations from the same animals, it is concluded that generalizations regarding changes in sensitivity to sympathomimetic amines following their prolonged administration should not be made in any one species.

Introduction

Between 1959 and 1966, mortality from bronchial asthma, particularly in younger age groups, increased three-fold in England and Wales (Speizer, Doll & Heaf, 1968). This has been attributed to excessive use of sympathomimetic bronchodilator agents administered by pressurized aerosol (Speizer, Doll, Heaf & Strang, 1968). In 72% of cases, the β-adrenoceptor stimulant used was isoprenaline.

Conolly, Davies, Dollery & George (1971) observed tolerance to the bronchodilator actions of isoprenaline *in vivo* in guinea-pigs. This was confirmed by Bouhuys, Douglas & Lewis (1972), but not by Pun, McCulloch & Rand (1971). Tolerance to the chronotropic actions of isoprenaline has also been reported as occurring in man and dogs (Conolly *et al.*, 1971) and in cats (Atkinson & Rand, 1968). More recently, however, Minatoya & Spilker (1975) did not observe tolerance to the chronotropic or bronchodilator actions of isoprenaline in *in vivo* experiments on healthy dogs. In a study of the ability of isoprenaline infusions to protect hypoxic dogs from the cardiotoxic effects of isoprenaline, McDevitt, Shanks & Swanton (1974) found no evidence of significant tolerance.

In a different approach, Benoy, El Fellah, Schneider & Wade (1975) observed that pretreatment of guinea-pigs three times daily with adrenaline given subcutaneously reduced the responsiveness to adrenaline of the isolated, perfused, histamine-constricted lungs of these animals. Moreover, they found that the longer the pretreatment period and the higher the dose of bronchodilator used in the pretreatment, the greater was the degree of tolerance developed.

The obvious controversy as to whether tolerance may develop in different tissues to β-adrenoceptor agonists might be resolved by studying quantitatively several actions of sympathomimetic bronchodilator drugs in tissues from the same animal after chronic

pretreatment with one of these drugs. In view of the fact that salbutamol, unlike adrenaline, is much more active on bronchial smooth muscle than on cardiac muscle (Cullum, Farmer, Jack & Levy, 1969; Farmer, Levy & Marshall, 1970; Daly, Farmer & Levy, 1971), it was of interest to observe whether tolerance would also develop in animals pretreated with salbutamol. Furthermore, since α-adrenoceptors have been shown to mediate contractions of bronchial smooth muscle (Takagi, Osada, Takayanagi & Taga, 1967; Everitt & Cairncross, 1969; Persson & Johnson, 1970) and adrenaline is a powerful agonist on both types of adrenoceptor, the possibility of the development of tolerance to α-adrenoceptor-mediated contractions of trachea was also investigated.

Methods

Animal pretreatment

(a) Attempted induction of β-adrenoceptor tolerance. A total of 36 albino guinea-pigs, of either sex and of 310–580 g body weight, were pretreated subcutaneously, three times daily at (09 h 00 min, 14 h 00 min and 20 h 00 min) for either 5 or 12 days with 0.9% w/v NaCl solution (saline), adrenaline (5 μg/kg) or salbutamol (0.5 μg/kg).
(b) Attempted induction of α-adrenoceptor tolerance. A total of 12 male guinea-pigs of 310–530 g body weight were pretreated subcutaneously, three times daily for 12 days with saline, adrenaline (5 μg/kg) or methoxamine (250 μg/kg).

In both pretreatment regimens, these doses were chosen on the basis of results obtained in experiments which showed that salbutamol was about ten times more potent and methoxamine about fifty times less potent than adrenaline in causing, respectively, relaxations and contractions of spiral preparations of trachea (Caswell, Cormack & Lees, unpublished observations). Dilutions of drugs were made up freshly with sterile saline before each pretreatment. The volume injected was 0.15–0.3 ml, depending on the body weight. Each animal showed a satisfactory and steady gain in weight throughout the pretreatment period. They were fed on a diet of Coney Brand pellets supplemented with greens and carrots three times a week; water, containing ascorbic acid (1 g/l), was available *ad libitum*.

Solutions and drugs

Isolated preparations were in contact with a physiological salt solution of the following composition (mM): NaCl 118.2, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25.0, glucose 11.2. The bath temperature was maintained at 37°C ± 0.5°C and the solution gassed with a mixture of 95% O_2 and 5% CO_2.

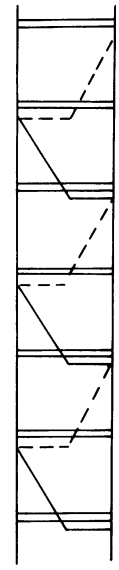

Figure 1 Diagrammatic representation of trachea showing position of cuts through muscle and cartilaginous rings. Solid lines indicate cuts on posterior surface of trachea, dashed lines show cuts on anterior aspect.

The following drugs were used: acetylcholine chloride (BDH); (−)-adrenaline (base) (Koch Light); isoprenaline hydrochloride (Winthrop); (±)-methoxamine hydrochloride (Burroughs Wellcome); (−)-noradrenaline acid tartrate (Koch Light); salbutamol (base) (Allan & Hanburys); (±)-propranolol hydrochloride (ICI); phentolamine hydrochloride or mesylate (Ciba).

Stock solutions of adrenaline were made up in 0.01 M HCl; acetylcholine stock solutions were prepared in 5% NaH_2PO_4. Other stock solutions were made up in a modified Krebs solution of the following composition (mM): NaCl 143, KCl 4.75, $CaCl_2$ 2.54. All were stored at −23°C. Dilutions of acetylcholine were acidified with HCl to bring the pH to 4. All other dilutions of stock solutions were made in the modified Krebs solution to which 0.2 mg/ml ascorbic acid was added to prevent oxidation. Final bath concentrations of all drugs are expressed in terms of the free base.

Preparations

The guinea-pigs were stunned by a blow on the head and bled from the left femoral artery. The heart was removed as quickly as possible and placed in warm Krebs solution. The atria were then carefully dissected from the ventricles and mounted in Krebs solution in an organ bath. A tension of 0.5 g was applied,

isometric contractions of the isolated atria being detected by strain gauge transducers (Ether, type UFl ± 2 ozs) and displayed on a Grass Polygraph (Model 7).

The trachea was carefully excised and cut spirally in the manner indicated in Figure 1: this method of cutting the trachea in steeper spirals results in an increased sensitivity. The tracheal spiral was mounted in Krebs solution at 37°C in an organ bath and a tension of 1.0 g applied. Isometric contractions and relaxations were recorded by means of strain gauge transducers connected to a Servoscribe (R.E. 250) potentiometric pen recorder. The wet weight of the tracheal strips, which was measured on completion of the experiment, was found to be 67.3 ± 1.3 mg (mean with s.e. mean; $n=49$).

The terminal portion of the ileum was used after the 20 cm nearest to the ileo-caecal junction had been discarded because of the presence of excitatory α-adrenoceptors near the ileo-caecal junction (Munro, 1953). The depressant actions of noradrenaline, isoprenaline, salbutamol and methoxamine were tested on 5–6 cm segments stimulated electrically with coaxial electrodes, essentially according to the method of Paton (1955). Isometric, twitch-like contractions of the ileum were recorded by means of a strain gauge transducer and displayed on a potentiometric pen recorder. The stimuli were 1.2–1.3 times maximal rectangular pulses of 0.4–0.5 ms duration at a frequency of 0.1 Hz; checks were made throughout an experiment to ensure that the stimuli were supra-maximal.

In all experiments, the preparations were allowed to recover for 1–1.5 h before starting the experiment. In the atria, dose cycles of not less than 20 min were employed in order to achieve consistent responses to adrenaline and isoprenaline and of not less than 30 min for salbutamol and methoxamine. In the trachea, dose cycles used for adrenaline and methoxamine were 25 min and 45 min respectively. All other agonists had dose cycles in this range. These times were based on the results of previous experiments.

Expression of results

Trachea: Since the resting tension was not constant but sometimes varied markedly, an allowance was made for these changes by measuring the change in muscle tension induced by a drug as a percentage of the tension existing immediately before exposure to the drug, i.e. these results are expressed as percentage loss or gain in tension.

Atria: Changes in rate and force of contraction were always expressed as percentage increases. After responses to salbutamol, however, the rate and contractile force never returned to the values observed before exposure to salbutamol. Thus, apparently smaller increases in heart rate and force of contraction were obtained because of the elevated 'background' values; an apparent bell-shaped dose-response relationship was frequently observed. The problem was overcome by assuming values of rate and contractile force prior to the first injection of salbutamol to be control values for the first and subsequent injections of salbutamol.

Ileum: The depressant action of drugs on the electrically-induced contraction of the longitudinal muscle was always expressed as percentage reduction of the twitch height before exposure to the drug (% inhibition of the twitch).

Statistical analysis

Decreases in responsiveness (tolerance) to adrenaline, salbutamol or methoxamine would appear as a rightward shift of the log dose-response curve compared with saline pretreated animals. In every case, the dose-response curves were analysed by Student's t test in order to determine whether a statistically significant displacement had occurred. Differences were taken to be significant when $P<0.05$, where P indicates the probability of two points being similar.

Results

β-Adrenoceptor-mediated responses in trachea

Adrenaline (10–300 nM), isoprenaline (3–300 nM) and salbutamol (1–300 nM) were found to induce relaxation of the preparations. There was no significant difference between the degree of relaxation induced by adrenaline in animals pretreated for 5 days with saline or adrenaline (Figure 2a). Pretreatment with adrenaline for 12 days, however, caused a significant displacement of the adrenaline dose-response curve to the right for the corresponding curve for trachea from saline-pretreated animals (Figure 2b). No statistically significant difference was observed between the relaxations induced by isoprenaline or salbutamol in adrenaline-pretreated and control animals ($P>0.1$) i.e. no significant cross-tolerance to these drugs was observed.

Responses to salbutamol in the trachea were unchanged after 5 and 12 days pretreatment with salbutamol as compared with saline-pretreated controls (Table 1 a & b). Cross-tolerance to adrenaline or isoprenaline was not observed.

β-Adrenoceptor-mediated responses in atria

Concentrations of adrenaline, isoprenaline and salbutamol were used which induced 10–60% increases in rate of beating; these concentrations

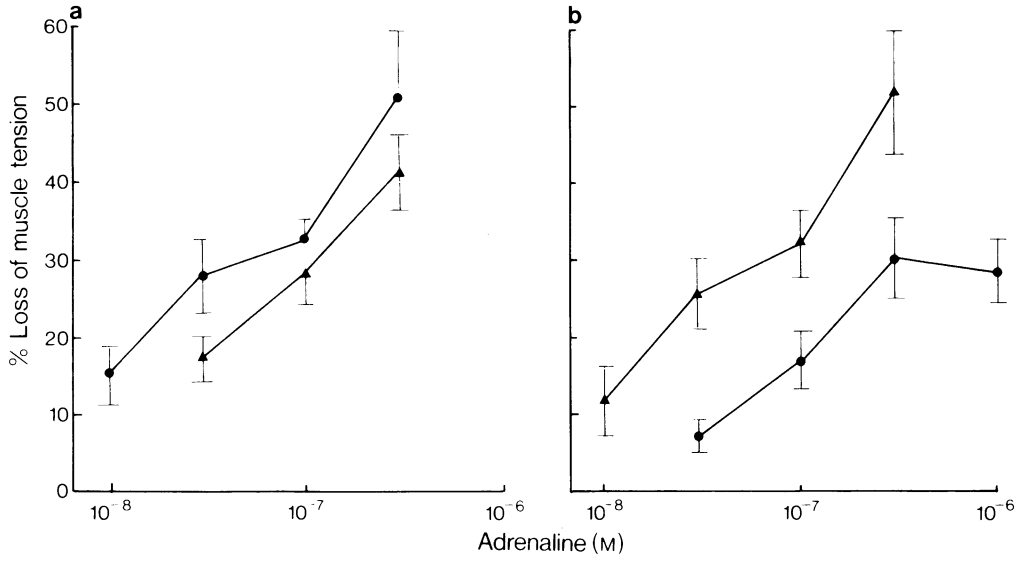

Figure 2(a) Relaxation induced by adrenaline in tracheal smooth muscle from guinea-pigs pretreated for 5 days with saline (▲) or adrenaline (●). Each point represents the mean and the vertical bars the s.e. mean of 6 observations for 6 animals; there were only 2 observations of responses to 300 nM adrenaline in adrenaline-pretreated animals. (b) Relaxation induced by adrenaline in tracheal smooth muscle from guinea-pigs pretreated for 12 days with saline (▲) or adrenaline (●) Points and vertical bars represent means with s.e. mean of 4–7 observations; there were only 2 observations of responses to 10 nM adrenaline in saline-pretreated animals. In (a) and (b) ordinates: loss of muscle tension (% control) (see Methods section: Expression of results); abscissae: concentration of adrenaline (M).

Table 1(a) Relaxation of tracheal smooth muscle by salbutamol after 5 day pretreatment with saline or salbutamol (0.5 μg/kg s.c.)

Salbutamol (nM)	*% Loss of bronchial muscle tension*		*Statistical analysis*	
	Saline-pretreatment	*Salbutamol-pretreatment*	t	P
1	11.3 ± 1.3(6)	—	—	—
3	16.2 ± 1.5(6)	14.1 ± 2.4(2)	0.74	0.49
10	25.8 ± 3.2(6)	27.9 ± 3.0(4)	−0.48	0.64
30	34.9 ± 10.1(2)	53.8 ± 2.7(6)	−1.81	0.10

Values are of mean with s.e. mean. The numbers in parentheses refer to the number of observations made in each case.

Table 1(b) Relaxation of tracheal smooth muscle by salbutamol after 12 day pretreatment with saline or salbutamol (0.5 μg/kg s.c.).

Salbutamol (nM)	*% Loss of bronchial muscle tension*		*Statistical analysis*	
	Saline-pretreatment	*Salbutamol-pretreatment*	t	P
1	13.2 ± 2.0(2)	—	—	—
3	22.2 ± 5.5(4)	12.8 ± 4.3(5)	1.35	0.23
10	29.5 ± 8.0(4)	19.3 ± 4.4(7)	1.12	0.29
30	33.6 ± 7.7(4)	30.7 ± 5.2(8)	0.31	0.76
100	—	31.9 ± 7.6(6)	—	—

Values are of mean with s.e. mean. The numbers in parentheses refer to the number of observations made in each case.

induced 25–400% increases in the force of isometric contractions of the atria. The relative potencies of adrenaline, isoprenaline and salbutamol were similar with respect to their ability to induce positive chronotropic and inotropic responses in the guinea-pig atria: isoprenaline was about 35 times more potent than adrenaline and about 450 times more potent than salbutamol.

In guinea-pigs pretreated with adrenaline for 12 days the chronotropic responses to adrenaline were not different from those of animals pretreated with saline for the same period ($P > 0.3$); similarly, the inotropic responses were unchanged ($P > 0.12$). In animals pretreated for 5 and 12 days with salbutamol, the chronotropic and inotropic responses to salbutamol were unchanged from those observed in atria from saline-pretreated controls ($P > 0.21$). As in the trachea, the phenomenon of cross-tolerance was not observed.

α-Adrenoceptor-mediated responses in trachea

Methoxamine has a direct α-adrenoceptor agonist action (Trendelenburg, 1972) and β-adrenoceptor blocking activity (Patil, Tye & La Pidus, 1967). In the concentration range 3–1000 nM, it induced slow, concentration-dependent contractions of the isolated tracheal strip, which were antagonized by phentolamine (300 nM). Acetylcholine (0.1–10 μM) also induced contractions of the trachea but these were much faster and were followed by a slower relaxation to less than basal tension; it required about 10 min for the smooth muscle tension to return to normal after these relaxations, which were not further investigated, attention being focussed on the contractions. Phentolamine (300 nM) was observed to potentiate the acetylcholine-induced contraction by $58.0 \pm 11.0\%$ (mean with s.e. mean, $n = 10$).

Contractor responses to methoxamine in four animals pretreated for 12 days with methoxamine or adrenaline were not significantly different from responses in saline-pretreated animals ($P > 0.05$). Pretreatment of animals for 12 days with either adrenaline or methoxamine did not modify the action of acetylcholine on the tracheal strip ($P > 0.12$).

α-Adrenoceptor-mediated responses in atria

Although there is no evidence that changes in rate and force of beating of the human heart are mediated by α-adrenoceptors, we found in guinea-pig isolated atria that methoxamine (1–300 μM) had a positive inotropic action but no positive chronotropic effect. This inotropic response was unaffected by propranolol (0.75 μM) but was abolished by phentolamine (300 nM), observations which are in agreement with the findings of Govier (1968). The chronotropic action of adrenaline (0.1–10 μM) was abolished and its inotropic action was greatly reduced by propranolol (0.75 μM): the remaining inotropic effect of adrenaline was abolished by phentolamine (300 nM). In saline-pretreated animals, methoxamine was about one thousand times less effective than adrenaline in inducing a positive inotropic action. To ensure that the inotropic responses to adrenaline and methoxamine were mediated purely by α-adrenoceptors and not partly by β-adrenoceptors, the responses were measured in the presence of a concentration of propranolol that was sufficient to block the chronotropic and inotropic actions of isoprenaline (100 nM), isoprenaline having a powerful action on β-adrenoceptors but almost no action on α-adrenoceptors. It was found that positive inotropic responses mediated by α-adrenoceptors were much smaller than those mediated by β-adrenoceptors. In the four guinea-pigs pretreated for 12 days with methoxamine or adrenaline there was, however, no unequivocal evidence that a lowered sensitivity had been induced.

α- and β-Adrenoceptor-mediated responses in ileum

The inhibitory effects of catecholamines on coaxially-stimulated guinea-pig isolated ileum are well documented and analysed (Kosterlitz, Lydon & Watt, 1970); both α- and β-adrenoceptors are involved in the depression of the twitch. This preparation was, therefore, chosen as a suitable one for examination of possible changes in responsiveness to sympathomimetic amines in the chronically pretreated guinea-pigs.

In confirmation of the findings of Kosterlitz *et al.* (1970), the minimum effective concentration of noradrenaline that produced a reduction of the twitch was 10 nM. Isoprenaline and noradrenaline, rather than adrenaline, were chosen as standard agonists because, in this preparation, noradrenaline has a pronounced β-adrenoceptor agonist action, whereas adrenaline acts predominantly on α-adrenoceptors (Kosterlitz *et al.*, 1970; Lees, unpublished observations). It had been assumed previously that isoprenaline produces an inhibition of the twitch due to an action on β-adrenoceptors. However, we found in some ileum preparations that, in the presence of propranolol (0.1–5 μM) there remained a small inhibitory effect which was reversed or prevented by phentolamine (0.1–2.5 μM); in some animals, therefore, up to about 25% of the inhibitory effect of isoprenaline is mediated by α-adrenoceptors. In the presence of isoprenaline or noradrenaline, in concentrations which produced maximal and half-maximal inhibition of the electrically-induced contractions, it was found that acetylcholine-induced contractions of the longitudinal muscle were depressed by these catecholamines and that this depression was partially reversed by phentolamine (0.1–0.5 μM) given

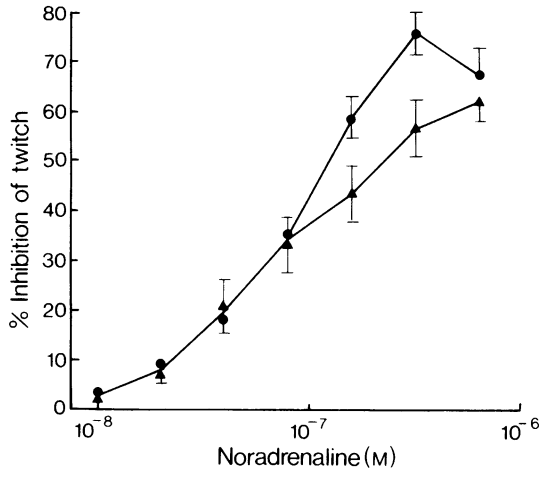

Figure 3 Depressant effect of noradrenaline on the responses to electrical stimulation (0.1 Hz) of the ileum from guinea-pigs pretreated for 12 days with saline (▲) or adrenaline (●). Ordinate scale: inhibition of contraction of longitudinal muscle (% control). Abscissa scale: concentration of noradrenaline (M). Points and vertical bars indicate means with s.e. mean of 7–12 observations; there were 3–4 observations of responses to 640 nM noradrenaline.

alone or during exposure to (−)-propranolol (1 μM). These results provide additional evidence that isoprenaline may act on α-adrenoceptors and that these receptors are probably present on the smooth muscle, as in the rabbit ileum (Bowman & Hall, 1970), in addition to being on neuronal elements (Kosterlitz *et al.*, 1970).

In animals pretreated with adrenaline for 5 and 12 days, the twitch of the longitudinal muscle was as greatly and as readily inhibited by noradrenaline as in the appropriate controls, with the exception of the response to the highest concentrations of noradrenaline (160 and 320 nM) which produced a significantly ($P < 0.05$) greater inhibition of the twitch of ilea from guinea-pigs pretreated with adrenaline for 12 days (Figure 3). This unexpected result is probably explained by the additional finding that the sensitivity to near maximally-effective concentrations of isoprenaline was significantly increased in these animals: the inhibition produced by 160 nM isoprenaline in saline controls was $14.3 \pm 2.9\%$ ($n = 8$), whereas in 12 day adrenaline-pretreated animals it was $24.1 \pm 3.3\%$ ($n = 11$) ($P < 0.05$). There were no significant changes in the sensitivity of the ileum to noradrenaline or isoprenaline in salbutamol- or methoxamine-pretreated guinea-pigs. Interestingly, salbutamol (0.64–5.12 μM) itself produced an inhibition of the longitudinal muscle contraction but it was at least 30 times less potent than isoprenaline. Methoxamine could not be successfully used as a standard α-adrenoceptor agonist in this preparation owing to the long-lasting dose-dependent contraction of the longitudinal muscle it induced; excitatory responses to noradrenaline were rarely seen and were very small.

Discussion

Over the last few years, studies of tolerance (i.e. a reduced responsiveness) to sympathomimetic bronchodilator drugs have been approached in two major ways, over short periods of only a few hours and over much longer periods, in clinical studies. However, in both of these approaches reports of whether tolerance develops or not in either man or experimental animals have been widely contradictory. More recently, Benoy *et al.* (1975) reported that tolerance to the bronchodilator action of adrenaline developed following pretreatment of guinea-pigs with adrenaline (5 μg/kg) for only three days. In an attempt to confirm and extend the findings of these authors, the same conditions of pretreatment were observed. The concentration of adrenaline which was used for pretreatment is approximately the same dose (on a weight per kilogram basis) as a patient with bronchial asthma might receive in an acute attack, so this must be regarded as a large dose when given regularly.

In the present experiments, salbutamol was found to be equipotent with isoprenaline in inducing relaxations of the tracheal muscle but 450 times less potent than isoprenaline in its chronotropic and inotropic actions on the isolated atria. Similarly, adrenaline was 10 times less potent than salbutamol in its action on the trachea but 13 times more potent on the atria. The results also indicate that tolerance to adrenaline in its action on bronchial smooth muscle developed after 12 days pretreatment but not after 5 days; in contrast, Benoy *et al.* (1975) observed tolerance after a much shorter pretreatment period and after 14 days pretreatment, the adrenaline dose-response curve was almost horizontal. The discrepency could be due to differences in experimental conditions; for example, Benoy *et al.* (1975) measured changes in the flow rate of saline solution in an isolated perfused lung preparation after administration of a bronchodilator drug, whereas, in the present study, relaxations of tracheal smooth muscle where measured. It is possible that drugs such as adrenaline, isoprenaline and theophylline have a more pronounced action on the muscle of the lower respiratory tract than of the upper respiratory tract. It is interesting that, although Benoy *et al.* (1975) reported cross-tolerance to adrenaline, isoprenaline and theophylline with respect to their bronchodilator actions, no evidence of cross-tolerance to isoprenaline or salbutamol was obtained in our ex-

periments. We did not observe tolerance to the action of salbutamol on tracheal smooth muscle even after 12 days pretreatment with salbutamol. This would suggest that, if a reduced sensitivity to the bronchodilator action of salbutamol can be induced, it develops more slowly than it does to adrenaline. In this connection, the results of a study by Sims (1974) in patients with reversible airways disease are of interest. These patients received either salbutamol orally for four weeks or salbutamol by aerosol inhaler for two weeks: the improvement in Forced Expiratory Volume in 1 s (FEV_1) induced by salbutamol in patients after these treatments was not significantly different from the improvement in FEV_1 recorded before treatment. In similar clinical studies, Gibson & Tattersfield (1972) and Parker, Choo-Kang, Cooper, Cameron & Grant (1971) did not observe any decrease in sensitivity following treatment with salbutamol, given orally, for four weeks.

Since Castro de la Mata, Penna & Aviado (1962) demonstrated the existence of α-adrenoceptors which were considered responsible for bronchoconstriction in the anaesthetized dog, much attention has been focussed on the existence of excitatory α-adrenoceptors in the respiratory tract. Evidence has been presented for the presence of these receptors in human bronchial musculature (Prime, Bianco, Griffin & Kamburoff, 1972; Gaddie, Legge, Petrie & Palmer, 1972). We were unable to demonstrate a decreased sensitivity to the α-adrenoceptor-mediated contractor response to methoxamine in tracheal smooth muscle in guinea-pigs pretreated for 12 days with adrenaline or methoxamine.

If it is assumed that responses mediated by the α-adrenoceptor, in addition to the β-adrenoceptor, play an important role in bronchial asthma, asthmatic patients may become tolerant to the bronchodilator action of adrenaline but not to the weaker bronchoconstrictor action. In this situation, the asthmatic condition would naturally be aggravated. This may be the basis for the improvement in airway conductance in asthmatic patients who have been given α-adrenoceptor blocking agents (Griffin, Kamburoff, Prime & Abbab, 1972).

Tolerance to the β-adrenoceptor actions of adrenaline or salbutamol on the isolated atria was not observed. This is in agreement with the results of clinical studies by Pierson & Grieco (1969), Cookson & Reid (1963) and Kingsley, Littlejohns & Prichard (1972) and of animal studies by Minatoya & Spilker (1975).

From the results obtained in the coaxially-stimulated ileum preparations, it is concluded that pretreatment of the guinea-pigs for 12 days with adrenaline or salbutamol did not lead to a diminished sensitivity of the ileum to β-adrenoceptor-mediated responses; indeed, there was evidence to suggest that the sensitivity was enhanced in the adrenaline-pretreated group, the tracheal muscle of these same animals showing a reduced responsiveness to the β-adrenoceptor-mediated relaxant action of adrenaline. In agreement with the findings for the trachea, there was no evidence of a changed sensitivity in the ileum to the α-adrenoceptor-mediated actions of noradrenaline in animals pretreated with adrenaline, methoxamine or salbutamol.

On the basis of our results obtained in three preparations each from the same guinea-pig, it is clear that generalizations regarding quantitative effects of sympathomimetic amines acting via β-adrenoceptors should not be made for any one species.

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