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SELECTIVE RESERPINE-INDUCED SUPERSENSITIVITY OF THE POSITIVE INOTROPIC AND CHRONOTROPIC RESPONSES TO ISOPRENALINE AND SALBUTAMOL IN GUINEA-PIG ISOLATED ATRIA

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1 Dose-response curves for the positive inotropic and chronotropic responses to isoprenaline were obtained in atria from untreated guinea-pigs and those receiving various reserpine pretreatments.

2 Tension responses were unaffected, whereas rate responses were depressed by the lowest dose of reserpine (0.05 mg/kg i.p. at 24 hours).

3 With larger 24 h doses and a 3 day pretreatment, the rate and tension dose-response curves were progressively displaced to the left, indicating supersensitivity which was greater for tension at each pretreatment.

4 No supersensitivity to histamine or Ca^{2+} could be detected, leading to the conclusion that it was selective for the β-adrenoceptor agonists possibly at the receptor level.

5 As an indication of the adrenergic neurone depleting effectiveness of each reserpine dosage, preparations were exposed to test doses of β-phenylethylamine.

6 Salbutamol was a partial agonist in untreated atria, the maximum rate (63.3%) and tension (10.0%) responses being less than those for isoprenaline. In atria from reserpine pretreated animals the supersensitivity was revealed as an increase of this maximum compared with isoprenaline.

7 The significance of this observation in relation to the possible mechanism of the supersensitivity is discussed.

Introduction

The adrenergic neurone-depleting activity of reserpine is accompanied by a gradual increase in sensitivity of the effector organ to catecholamines (Fleming & Trendelenburg, 1961). This supersensitivity has been compared with that following decentralization of the organ since they have been found to exhibit similar characteristics of a slow onset of action and relative non-specificity (Trendelenburg, 1963). There are however many conflicting reports of failure to induce supersensitivity by reserpine, particularly in isolated tissues taken from pretreated animals. This is illustrated by the nictitating membrane, for which there are numerous demonstrations of supersensitivity in the intact animal (Trendelenburg & Weiner, 1962; Fleming, 1963; Seidehamel, Patil, Tye & LaPidus, 1966). Isolated preparations however have been shown to lack the enhanced sensitivity (Tsai, Denham & McGrath, 1968). Similarly cardiac muscle has shown discrepancies between intact and isolated hearts. Supersensitivity to the positive chronotropic effects of noradrenaline has been found in open-chest dogs (Trendelenburg & Gravenstein, 1958), and similar reports have been made for dog heart-lung preparations (Bejrablaya, Burn & Walker, 1958; Westfall & Fleming, 1968a) and guinea-pig hearts *in vivo* (Westfall & Fleming, 1968b). Potentiation of the rate responses to noradrenaline by reserpine-treatment has been shown in perfused hearts (Westfall & Fleming, 1968b), but not in guinea-pig isolated atria (Crout, Muskus & Trendelenburg, 1962; Westfall & Fleming, 1968b). The latter authors, like Tsai *et al.* (1968) for the nictitating membrane, attributed this failure to demonstrate supersensitivity to the degree of trauma on removal from the animal.

The positive inotropic responses to noradrenline have received less attention, being studied exclusively in isolated perfused hearts where McNeill & Schulze (1972) have reported satisfactory sensitization.

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Taylor, Westfall & Fleming (1974) on the other hand, claim that it is only the chronotropic effects that are enhanced by reserpine, and any potentiation of the inotropic responses is due to the influence of the rate changes upon the tension in spontaneously beating hearts.

In view of these conflicting reports of supersensitivity between the *in vivo* and *in vitro* hearts and the possible differences between the positive inotropic and chronotropic responses to catecholamines, we have examined the effects of reserpine pretreatment upon the responses to isoprenaline in our experimental model. This consists of separated left and right guinea-pig atria; the spontaneously beating right atrium provides the rate responses and the left atrium is driven at a constant rate to provide the tension responses. This avoids any effects of rate changes upon the tension responses (Koch-Weser & Blinks, 1963). A preliminary report of this work has been presented to the British Pharmacological Society (Broadley & Lumley, 1975).

Methods

Guinea-pigs of either sex and weight range 400–600 g were killed by a blow on the head and exsanguinated under running water. The thorax was rapidly opened and the right and left atria removed separately. The right atrium was removed first, with cotton loops tied through the inferior vena cava and the atrio-ventricular junction which secured it to the perspex tissue holder (Blinks, 1966); a third cotton thread was passed through the superior vena cava for connection to an isometric transducer (Ether, Type UF 1, 28 g sensitivity range). To the left atrium was sewn an L-shaped stud which clamped it onto the tissue holder in close contact with a pair of punctate platinum electrodes. A cotton thread through the tip of the left atrial appendage passed to a second isometric transducer.

The atria, attached to the tissue holder, were immersed in an organ bath (50 ml) containing Krebs-bicarbonate solution having the following composition in g/l distilled water; NaCl 6.92, KCl 0.345, $CaCl_2 2H_2O$ 0.28, $NaHCO_3$ 2.1, $MgSO_4 7H_2O$ 0.29, glucose 2.0, $NaH_2PO_4 2H_2O$ 0.16. This was gassed with 5% CO_2 in O_2 and maintained at a temperature of 38 ± 0.5°C. Initial resting diastolic tensions of 1 and 0.5 g were applied to the left and right atria respectively. Records of force of contraction were obtained from the left atrium driven at a constant rate of 2.5 Hz with square wave pulses (5 ms and threshold voltage plus 50%) delivered by an SRI stimulator (Type 6053) and the tension developed was recorded on a Devices M19 polygraph. Rate of contraction was recorded from the spontaneously beating right atrium by means of a ratemeter (Devices, Type 2751) triggered by the tension signal.

After a 30 min stabilization period for the rate and tension to become constant, during which several changes of bathing medium were made, a preliminary cumulative dose-response curve to the agonist was obtained. The concentration was increased ten-fold until the maximum response was recorded. No difference in maximum response was found between cumulative and sequential administration of isoprenaline. After the tissue had been washed and the tension and rate had returned to their pre-drug levels, a second cumulative dose-response curve was constructed by increasing the concentration three-fold. Since it has been the experience of ourselves and others (Levy, personal communication) that the first dose-response curve to isoprenaline in guinea-pig atria is dissimilar in slope to subsequent curves, only the second, more detailed curve, was used for plotting purposes and calculation of the EC_{50}. The agonists under study were (−)-isoprenaline, histamine and calcium, but only with isoprenaline was a preliminary curve obtained. In some experiments with isoprenaline as the agonist, a third cumulative dose-response curve to salbutamol was obtained and comparisons were made between this and the second isoprenaline curve. The isoprenaline curves plotted in these experiments were corrected for any differences in sensitivity between the second (isoprenaline reference) and third (salbutamol) curve by performing control experiments in which three isoprenaline dose-response curves were obtained on the tissue. The mean difference in response size at each concentration was expressed as a percentage and this factor applied to the individual second isoprenaline curves. This predicted the sensitivity of the preparation to isoprenaline had the curve been obtained at the same point in time as the salbutamol curve.

Experiments were performed with atria from untreated animals and those pretreated with reserpine. The reserpine-induced supersensitivity was examined by using several pretreatment schedules; (a) 0.05 mg/kg (i.p.) at 24 h, (b) 0.5 mg/kg at 24 h, (c) 5 mg/kg at 24 h and (d) 5 mg/kg at 72 h, 3 mg/kg at 48 h and 3 mg/kg at 24 h before the animals were killed. A measure of the level of depletion of noradrenaline caused by these reserpine-treatment schemes was obtained by exposing the preparations to a single dose (0.8×10^{-4}M) of the indirectly acting sympathomimetic amine β-phenylethylamine (Burn & Rand, 1958) before starting the experiment at the end of the stabilization period. The rate and tension responses were compared with cumulative dose-response curves for this amine produced in atria from untreated animals.

Drugs used were: histamine acid phosphate (Sigma), (−)-isoprenaline bitartrate dihydrate (Ward Blenkinsop Ltd), β-phenylethylamine as the base (Koch-Light Laboratories), reserpine (BDH Ltd) and salbutamol (Allen & Hanburys Ltd). All solutions were freshly prepared in 0.9% w/v NaCl solution

(saline) with the exception of reserpine which was dissolved in 20% ascorbic acid and 1 N NaOH giving a solution with a pH of 5.0.

Results

The rate responses of the right atria and the tension responses of the left atria were measured as the increase above the pre-drug level at each concentration. The mean rate and tension increases of at least six preparations were then determined. To facilitate comparison of rate and tension on the same graph, the mean response sizes were expressed as a percentage of the maximum response and plotted as such. Assessment of any supersensitivity was made by comparing the mean molar EC_{50} values for rate and tension between atria from untreated guinea-pigs and those receiving the various pretreatments. Geometric means, calculated as the mean of the logarithm of the individual EC_{50} values, were compared statistically by Student's *t*-test, the results of which are presented in Table 1 with the geometric means and their 95% confidence limits.

Isoprenaline in untreated atria was rate-selective in that the rate curve was to the left of that for tension. The mean curves of 39 experiments are plotted in each part of Figure 1 for comparison with the rate and tension curves obtained in atria from animals receiving the four pretreatment schedules. Schedule (a), the lowest dose of reserpine used, produced no change in the tension curve but depressed the rate responses (Figure 1a) as shown by a significant ($P<0.005$) displacement of the EC_{50} to the right (Table 1). On increasing the reserpine dose to 0.5 mg/kg in schedule (b), supersensitivity of the tension responses to isoprenaline was revealed as a shift of the curve to the left (Figure 1b) with a significant ($P<0.001$) reduction of the EC_{50} (Table 1). The rate responses were no longer depressed but there was no sign of any supersensitivity. Further increase of the 24 h dose to 5.0 mg/kg in schedule (c) produced supersensitivity of both rate and tension responses and although the rate curve was shifted only slightly to the left, the fall in EC_{50} value was significant ($P<0.01$). The tension curve however was displaced no further than with schedule (b), having an identical EC_{50} value. When the reserpine-treatment programme was extended to three days there was an additional shift of the tension dose-response curve giving an EC_{50} value of 2.2 nM compared with 13.4 nM in untreated atria. The rate curve was similarly moved more to the left than with the 24 h treatment. Therefore, as the severity and duration of the reserpine pretreatment increased so the rate and tension responses were progressively potentiated; however, the tension curves were apparently displaced to a greater extent thus tending to close the rate-tension difference seen in untreated atria. The dose-ratios for rate and tension geometric mean EC_{50} values were 5.1 and 3.4 in untreated and three day pretreated atria respectively.

Since atria from untreated animals were used as the reference for the reserpine-treatment programmes, a check was made that any changes in sensitivity were not merely due to the injection procedure and the vehicle, which proved to be slightly irritant. A group of animals were therefore injected for three days with the reserpine vehicle and the positions of the iso-

Table 1 Mean EC_{50} values for rate and tension responses to isoprenaline and histamine of atria from untreated and reserpine-pretreated guinea-pigs

	Isoprenaline (nM)		*Histamine (μM)*	
Reserpine pretreatment	*Rate*	*Tension*	*Rate*	*Tension*
Nil	2.6 (2.2–3.2)	13.4 (11.6–15.5)	1.15 (0.72–1.81)	1.24 (0.53–2.95)
0.05 mg/kg i.p. at 24 h	5.3† (3.5–8.0)	11.1^{NS} (7.7–15.8)	—	—
0.5 mg/kg i.p. at 24 h	3.2^{NS} (1.2–8.2)	5.0* (3.2–7.9)	0.97^{NS} (0.72–1.31)	1.37^{NS} (0.88–2.13)
5.0 mg/kg i.p. at 24 h	1.5‡ (1.2–1.9)	5.0* (3.4–7.4)	1.32^{NS} (0.86–2.03)	0.83^{NS} (0.64–1.08)
5.0 mg/kg at 72 h and 3.0 mg/kg i.p. at 48 and 24 h	0.64* (0.41–0.99)	2.2* (1.4–3.4)	0.86^{NS} (0.59–1.25)	1.4_0^{NS} (1.05–2.02)

Geometric means and their 95% confidence limits are shown.
Significance levels for differences in EC_{50} values between untreated and reserpine-pretreated atria determined by Student's *t*-test are represented by; NS, not significant; * $P<0.001$; † $P<0.005$ and ‡ $P<0.01$.

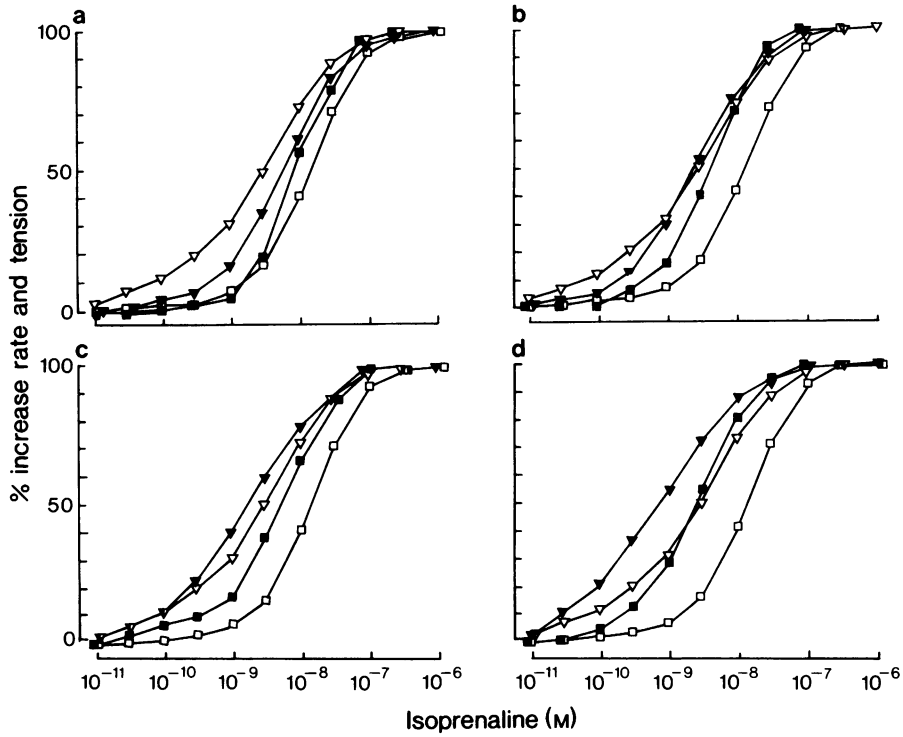

Figure 1 Mean ($n=39$) cumulative dose-response curves plotted as a percentage of the maximum response for the increase in rate (▽) and increase in tension (□) responses to isoprenaline of isolated atria from untreated guinea-pigs. These are compared with the mean rate (▼) and tension (■) dose-response curves in atria from guinea-pigs pretreated with reserpine: (a) 0.05 mg/kg i.p. at 24 h ($n=10$), (b) 0.5 mg/kg i.p. at 24 h ($n=7$), (c) 5.0 mg/kg i.p. at 24 h ($n=10$), and (d) 5.0 mg/kg i.p. at 72 h and 3.0 mg/kg at 48 and 24 h before guinea-pigs were killed ($n=15$).

prenaline dose-response curves obtained in the atria from these animals (EC_{50} values for rate 4.0 nM and tension 12.6 nM) were not significantly different from those from untreated animals (EC_{50} values for rate 2.6 nM and tension 13.4 nM). The vehicle and injection procedure were therefore not responsible for the sensitivity changes encountered.

The supersensitivity has so far been measured with dose-response curves plotted as a percentage of the maximum response, where any changes in resting levels or absolute maximum reponses may be masked. However, a check on the reserpine pretreatments shown to induce supersensitivity to isoprenaline revealed resting rates of 245.7 ± 5.8, 245.4 ± 6.6 and 264.9 ± 5.3 beats/min after the 0.5 mg/kg, 5.0 mg/kg and three day pretreatments respectively compared with 235.5 ± 3.5 beats/min in untreated atria. The resting developed tensions after these pretreatments were 0.56 ± 0.06, 0.59 ± 0.06 and 0.78 ± 0.08 g respectively compared with 0.57 ± 0.03 g in the untreated atria. Therefore only after the three day pretreatment was there any significant difference ($P<0.05$). The maximum absolute rate increases did not vary significantly between the three day and the 0.05, 0.5 and 5 mg/kg 24 h reserpine pretreatments (103.2 ± 5.5, 116.6 ± 7.2, 114.9 ± 7.4 and 119.6 ± 5.9 beats/min respectively). Similarly the absolute tension increases at the maximum did not differ significantly between the three day and 24 h pretreatments (0.69 ± 0.06, 0.66 ± 0.14; 0.72 ± 0.09 and 0.82 ± 0.13 g respectively).

Having satisfactorily demonstrated supersensitivity of both rate and tension responses to isoprenaline in our model, we continued with an examination of its selectivity by first obtaining dose-response curves to histamine in atria from untreated and reserpine pretreated animals receiving schedules (b), (c) and (d). Unlike isoprenaline, histamine was equiactive on rate and tension in untreated atria and these, almost superimposable dose-response curves, were compared with those from the pretreated atria (Figures 2a, b and c). The rate and tension dose-response curves remained virtually unaltered after each of the pretreatments. There was a slight shift to the left of the tension curve

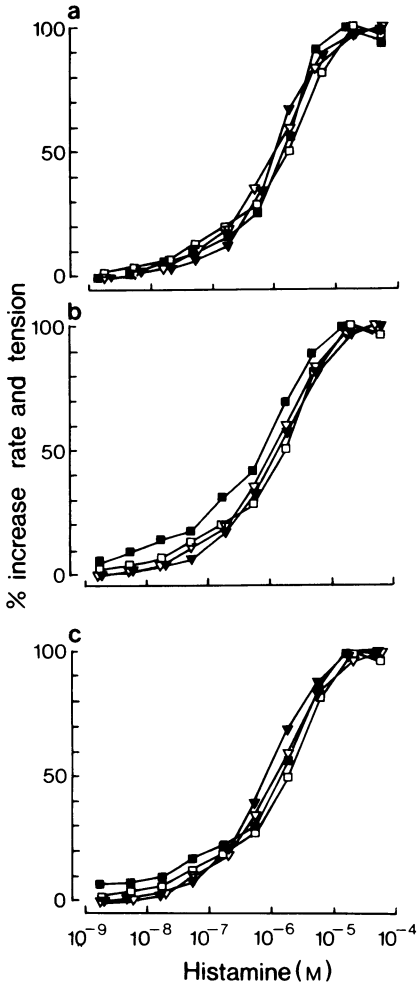

Figure 2 Mean ($n=10$) cumulative dose-response curves plotted as a percentage of the maximum response for the increase in rate (▽) and increase in tension (□) responses to histamine of isolated atria from untreated guinea-pigs. These are compared with the mean rate (▼) and tension (■) dose-response curves in atria from guinea-pigs pretreated with reserpine: (a) 0.5 mg/kg i.p. at 24 h ($n=6$), (b) 5.0 mg/kg i.p. at 24 h ($n=6$), and (c) 5.0 mg/kg i.p. at 72 h and 3.0 mg/kg at 48 and 24 h before guinea-pigs were killed ($n=6$).

after the 5 mg/kg dose of reserpine but the EC_{50} value of this, and all the curves from pretreated atria, was not significantly different from the untreated atria values. Any small changes in position of the curves therefore could be attributed to scatter of the results.

The apparent selectivity of the supersensitivity for isoprenaline was further tested by examining $CaCl_2$, in this case only in untreated atria and those following the three day pretreatments schedule (d) (Figure 3).

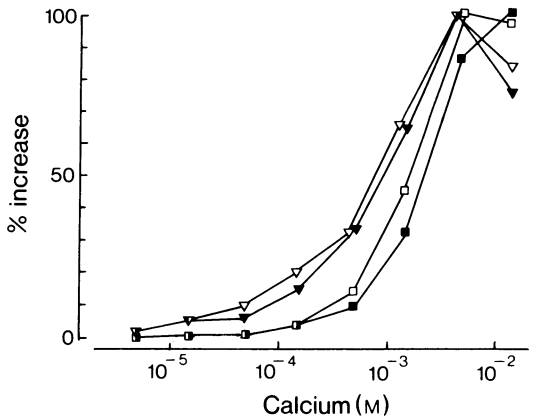

Figure 3 Mean ($n=4$) cumulative dose-response curves plotted as a percentage of the maximum response for the increase in rate (▽) and increase in tension (□) responses to Ca^{2+} (calcium chloride) of isolated atria from untreated guinea-pigs. These are compared with the mean ($n=4$) rate (▼) and tension (■) dose-response curves in atria from guinea-pigs pretreated with reserpine 5.0 mg/kg i.p. at 72 h and 3.0 mg/kg at 48 and 24 h before they were killed.

This, the most effective pretreatment for inducing supersensitivity to isoprenaline, failed to alter the dose-response curves to calcium. If anything the tension curve was slightly depressed.

During the course of the study so far, each preparation had routinely received a test dose of the indirectly acting sympathomimetic amine β-phenylethylamine before starting the first dose-response curve. The rate and tension increase responses for each pretreatment were collected and the mean values compared with a full dose-response curve obtained in untreated atria (Figure 4). The selected dose produced approximately 50% of the maximum tension and almost a maximum rate response in untreated atria. After the three schedules (b), (c) and (d) the rate response was almost abolished, although the tension response was only halved. These responses were almost identical in the three groups of atria so that the three day pretreatment did not apparently have any further effect than the 24 h pretreatment. However, after the lowest dose of reserpine (0.05 mg/kg) the β-phenylethylamine responses were reduced to a lesser extent than by the higher doses.

Salbutamol is a partial agonist at the β_1-adrenoceptors of the heart (Brittain, Jack & Ritchie, 1970) and offered the opportunity to examine an alternative aspect of the reserpine-induced supersensitivity already demonstrated for isoprenaline; namely the effect upon the maximum response to this sympathomimetic amine. Salbutamol and isoprenaline dose-response curves were compared in the same preparations from untreated guinea-pigs or

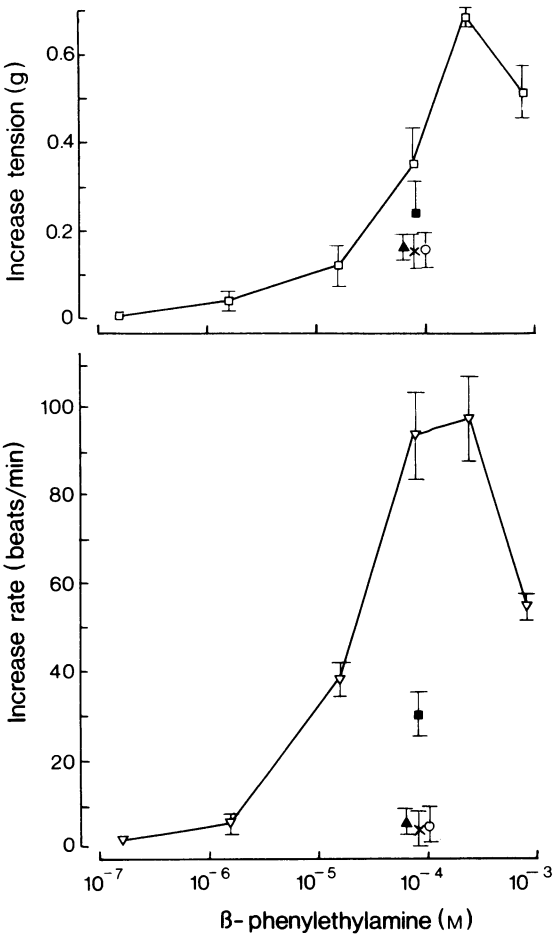

Figure 4 Mean cumulative dose-response curves for the increase in tension (above, □) and increase in rate (below, ▽) responses to β-phenylethylamine in atria from untreated guinea-pigs. Each point represents the mean of three experiments. Vertical lines show s.e. mean. The single symbols represent the mean responses (± s.e.) to the test dose (0.8×10^{-4} M) of β-phenylethylamine in atria from guinea-pigs receiving reserpine 0.05 mg/kg i.p. at 24 h (■, $n=8$), 0.5 mg/kg i.p. at 24 h (▲, $n=18$), 5.0 mg/kg i.p. at 24 h (×, $n=9$) and 5.0 mg/kg i.p. at 72 h and 3.0 mg/kg at 48 and 24 h (○, $n=8$) before they were killed.

animals pretreated with 5 mg/kg reserpine (Figure 5) and the three day pretreatment (Figure 6), the salbutamol curves being plotted as a percentage of the isoprenaline maximum. In untreated atria, the maximum tension response to salbutamol was only $10.0 \pm 1.1\%$ ($n=4$) that of isoprenaline, although it was more of a full agonist on rate at $63.3 \pm 3.4\%$ of the isoprenaline maximum. However, in atria from animals receiving 5 mg/kg, the rate and tension maxima were raised to $72.6 \pm 5.1\%$ ($n=4$) and $24.9 \pm 6.0\%$ of the isoprenaline maxima respectively (Figure 5). After the three day pretreatment there was a further elevation of the maximum responses to $77.7 \pm 4.1\%$ ($n=3$) and $44.7 \pm 5.7\%$ ($n=4$) respectively (Figure 6). The salbutamol dose-response curves were also visibly shifted to the left by the reserpine pretreatment similarly to the isoprenaline curves. The three day pretreatment of Figure 6 in particular demonstrates the preferential reserpine-induced supersensitivity for tension responses, with the rate and tension dose-response curves to isoprenaline having moved closer.

Discussion

The isolated left and right atria of the guinea-pig have been clearly shown to exhibit supersensitivity to isoprenaline after a range of reserpine pretreatments. This observation confirms previous reports of an enhanced sensitivity of both the positive inotropic (McNeill & Schulze, 1972) and chronotropic responses (Westfall & Fleming, 1968b; Taylor *et al.*, 1974) to catecholamines in isolated cardiac preparations. However, it does not conform to the opinion of Westfall & Fleming (1968b) that excessive trauma or dissection of the preparation obscures any supersensitivity, Neither they using a bath temperature of 38°C, nor Crout *et al.* (1962) using 32°C, could demonstrate sensitization of isolated atria. The dissection employed here was perhaps more extensive than that employed by these groups of workers. Although Westfall & Fleming (1968b) demonstrated some supersensitivity to the inotropic responses in perfused hearts, they have more recently proposed (Taylor *et al.*, 1974) that this is only apparent and due to concomitant rate increases that are known to exert positive inotropic effects themselves (Koch-Weser & Blinks, 1963). In perfused hearts electrically paced at a constant rate, they found no supersensitivity to the inotropic effects of noradrenaline. Our observations contradict this finding since they too were obtained in a paced preparation and unlike Taylor *et al.* (1974), the supersensitivity was apparent after only 24 h pretreatment and increased with extension of the pretreatment period. A possible explanation for the failure of Taylor *et al.* (1974) to find supersensitivity is the fact that they paced their hearts at 60 beats/min above resting rates. In our experience guinea-pig atrial preparations have resting rates of about 240 beats/min (238 ± 3.1, $n=70$) at 38°C. At high frequencies the availability of immediate sources of energy may become a limiting factor in the amount of tension which may be developed so that drugs like acetylstrophanthidin produce little positive inotropic effect (Koch-Weser & Blinks, 1962). A frequency of about 5 Hz, as used by Taylor *et al.* (1974), could thus

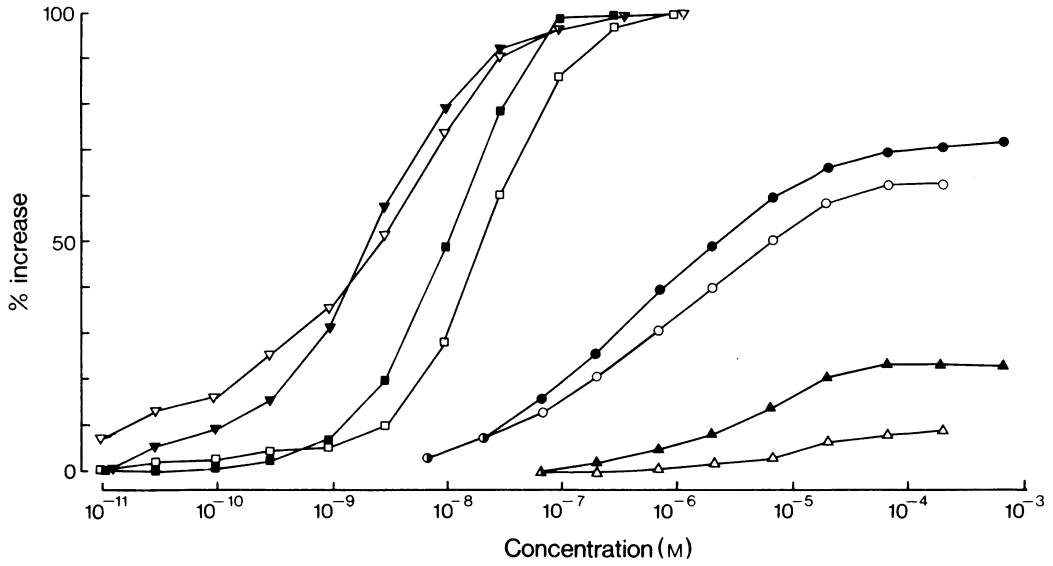

Figure 5 Mean ($n=4$) cumulative dose-response curves to isoprenaline (increase rate, ▽; increase tension, □) and salbutamol (increase rate, ○; increase tension, △) plotted as a percentage of the isoprenaline maximum response in isolated atria from untreated guinea-pigs. These are compared with the mean ($n=4$) dose-response curves to isoprenaline (rate, ▼; tension, ■) and salbutamol (rate, ●; tension, ▲) obtained in atria from guinea-pigs pretreated with reserpine 5.0 mg/kg i.p. at 24 h before they were killed.

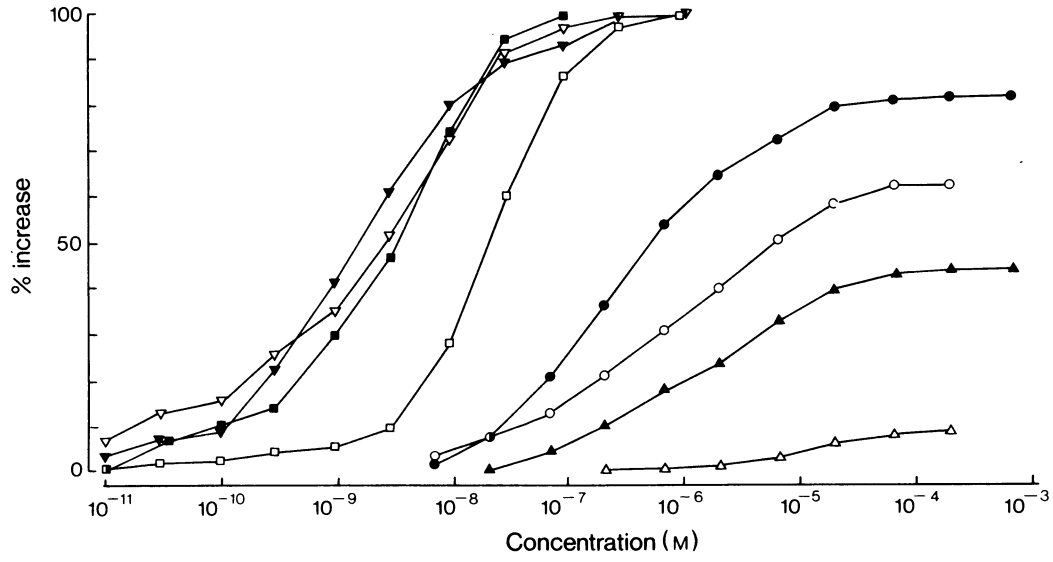

Figure 6 Mean ($n=4$) cumulative dose-response curves to isoprenaline (increase rate, ▽; increase tension, □) and salbutamol (increase rate, ○; increase tension, △) plotted as a percentage of the isoprenaline maximum response in isolated atria from untreated guinea-pigs. These are compared with the mean ($n=3$) dose-response curves to isoprenaline (rate, ▼; tension, ■) and salbutamol (rate, ●; tension, ▲) obtained in atria from guinea-pigs pretreated with reserpine 5 mg/kg i.p. at 72 h and 3.0 mg/kg at 48 and 24 h before they were killed.

affect the inotropic action of the catecholamines and possibly mask any supersensitivity.

The sensitivity of the atria to the tension responses did not increase between the 0.5 and 5 mg/kg dose schedules, indicating perhaps that there is a maximum supersensitivity that may be achieved with 24 h pretreatment and that further increases in reserpine dose are without further effect. However, prolonging the pretreatment period did cause additional increase in sensitivity. This agrees with the findings of many other studies that the dosage and more particularly, the time of exposure to reserpine are important for supersensitivity (Fleming, McPhillips & Westfall, 1973). The importance of time of exposure has been stressed and most workers have found 24 h to be insufficient, although the cardiovascular system appears to become supersensitive more rapidly (Fleming & Trendelenburg, 1961), thus possibly explaining our results.

The positive chronotropic responses did not follow the same pattern. At the lowest dose of reserpine there was in fact depression of the rate responses to isoprenaline. Antagonism of sympathomimetic responses in rat atria following chronic administration of reserpine has been reported previously (Tiwari, Joshi, Inamdar & Sadre, 1966) and may be equivalent to our observation which would appear to be selective only for the pacemaker activity controlling rate. Only at the 5 mg/kg dose was there a significant potentiation of the rate responses and thereafter further potentiation occurred after the three day pretreatment. The supersensitivity was preferential for the tension responses at each of the reserpine-treatment schedules. This may be due to the depression which was seen only on the rate reponses with the 0.05 mg/kg dose counteracting any supersensitivity at the other pretreatment schedules. This subsensitivity may also increase with reserpine dose, thus preventing the supersensitivity for rate from achieving the same magnitude as that for tension. Alternatively the supersensitivity may be genuinely preferential for tension.

The question of the selectivity of the supersensitivity only for sympathomimetic amines now arises. The previously reported supersensitivity to the positive inotropic effects of noradrenaline in perfused hearts was accompanied by an increased sensitivity to histamine (McNeill & Schulze, 1972) and the positive chronotropic supersensitivity by an enhanced response to calcium (Westfall & Fleming, 1968b). On this evidence the supersensitivity of the heart was proposed to be non-specific. The present results are at variance with these views since neither the histamine nor calcium dose-response curves were displaced by the reserpine pretreatments. Our preliminary studies (Broadley & Lumley, 1975) described one instance of a shift of only the tension curve of histamine to the left following the 5 mg/kg 24 h pretreatment. However, further work with larger groups and with the three day pretreatment has shown that this result was erroneous and no supersensitivity to histamine could be detected with any of the pretreatments.

In tissues other than the heart there is more abundant evidence that the supersensitivity may be non-specific (Fleming *et al.*, 1973). However, there are also many reports of failure to induce supersensitivity to other agonists. For example the lack of supersensitivity to histamine in the aortic strip led Hudgins & Fleming (1966) to classify the supersensitivity as relatively non-specific.

In view of the divergence of our findings from previously held opinions, we were concerned that they could not be invalidated by the methods of expression of our results. As a quantitative measure of the degree of supersensitivity we have used the horizontal displacement of dose-response curves. Mean EC_{50} values were compared statistically by the use of geometric means which are more valid than arithmetic means for examining sensitivity differences (Fleming, Westfall, De La Lande & Jellett, 1972). To obtain EC_{50} values necessitated plotting the curves as a percentage of the maximum response. Kalsner (1974) has warned against the sole use of this method especially when the resting levels and absolute maxima may change after reserpine pretreatment. In the present work, the supersensitivity was observed in atria whose resting levels did not change. Similarly the absolute rate and tension increases at the maxima were of the same order after the various pretreatment schedules and therefore the progressive supersensitivity was not a function of the plotting method.

Finally, the possible mechanism of the sensitization of guinea-pig atria to isoprenaline, assuming it to be selective, must be considered. The supersensitivity has been causally related to the level of depletion of noradrenaline which thus interrupts the passage of nervous impulses to the organ, however depletion *per se* is not sufficient to induce supersensitivity since an additional time factor is also required (Fleming & Trendelenburg, 1961). In the present study a measure, albeit very limited, of neuronal depletion was obtained by recording the responses to the indirectly-acting sympathomimetic amine β-phenylethylamine in atria from each reserpine-treatment schedule. These were only partially prevented by the 0.05 mg/kg 24 h dose of reserpine which therefore could tentatively be concluded to have failed to deplete the noradrenaline satisfactorily; it also failed to induce supersensitivity. The subsequent dosage regimens all abolished rate responses indicating total depletion in the region of the SA-node, whereas the tension responses were only halved. By this test, depletion in the left atrium and myocardium could therefore be assumed to be incomplete and the 24 h pretreatment could not be bettered by three day exposure; it was presumably maximal. The noradrenaline stores of the left atrium therefore appear to be more resistant to depletion than those of the SA-node, which agrees with the findings of Blinks (1966). These results would suggest that the

supersensitivity and depletion are not directly related since there was maximal depletion after the 0.5 mg 24 h dose, although the supersensitivity continued to develop with the dose (rate) and time of exposure (tension). It is also possible to obtain some depletion without supersensitivity. To achieve optimum depletion with minimal supersensitivity, the 24 h 0.5 mg/kg dose would appear to be suitable. To substantiate these claims, noradrenaline concentrations in the left and right atria would provide a better index of depletion.

Although in the past a prejunctional noradrenaline binding phenomenon was implicated in the supersensitivity (Kirkpekar, Cervoni & Furchgott, 1962), in recent years a postjunctional mechanism has been suggested with the non-selectivity lying at the cell membrane level (Trendelenburg, 1963; Fleming *et al.*, 1973). The present work has demonstrated sensitization to only sympathomimetic amines suggesting a more limited site of action than the common cellular pathways, possibly at the receptor itself. A change in the nature of the receptor can be discounted from a previous observation (Lumley & Broadley, 1975) that the pA_2 values of practolol are identical in atria from untreated guinea-pigs and those receiving the three day reserpine-treatment schedule used here. This agrees with findings of no change in affinity of phentolamine and phenylephrine for α-adrenoceptors in aortic strips (Taylor & Green, 1971) and the nictitating membrane (Green & Fleming, 1967). The latter workers however found that the non-competitive antagonism of noradrenaline by phenoxybenzamine was reduced after 14 days of reserpine pretreatment. This was explained by either a change in the relationship between receptor occupation and response or by an increase in receptor numbers. A greater number of available receptors would permit noradrenaline to produce a maximum response in the presence of higher concentrations of phenoxybenzamine. The fact that pA_2 values remain constant does not however oppose the concept of receptor proliferation since they are derived from equal responses in the presence and absence of the antagonist involving the same number of receptors (Arunlakshana & Schild, 1959). They are therefore independent of the total receptor population (Green & Fleming, 1967).

The results obtained here with salbutamol may help to resolve this problem. The observation that the rate and tension responses to salbutamol fail to attain the same maximum as isoprenaline agrees with others (Brittain *et al.*, 1970; Hughson & Ledsome, 1975) and permits its classification as a partial agonist at the β-adrenoceptors of the heart (Stephenson, 1956). It can therefore be assumed to occupy all the available receptors when producing this reduced maximum response (Stephenson, 1956). Reserpine-treatment increased the maximum response compared with that of isoprenaline, but did not convert salbutamol to a full agonist. Presumably it therefore still occupied all the receptors, but the larger response might indicate that the number of receptors had increased. The rate response maximum to salbutamol was greater in each group of atria than the corresponding tension response maximum, which might also suggest that different numbers of receptors are involved in the right and left atria. This may be an alternative explanation for the preferential potentiation of the tension responses to isoprenaline by reserpine. If there were a greater receptor density in the right atrium than in the left atrium, which is possible in view of its denser innervation (Angelakos, Fuxe & Torchiana, 1963), then with each dose of isoprenaline a greater proportion might be occupied in the left atrium. Thus there are fewer spare receptors (Stephenson, 1956) and receptor proliferation might be expected to have a more marked effect upon the response size than in the right atrium where there may be more spare receptors and an increase in total receptor population would have proportionately less effect. This situation is analogous to non-competitive antagonism whereby the antagonist is irreversibly attached to a certain proportion of the receptors so effectively reducing the number of receptors, leading to a reduction of the maximum response. Thus changes in the total number of receptors can affect the size of the maximum response.

The raised maxima to salbutamol by reserpine may alternatively be explained not by receptor proliferation, but by an increased efficacy, whereby the same receptor occupation and affinity would generate a larger response. In fact Taylor & Green (1971) derived an equation that predicted an increased efficacy for phenylephrine in the aortic strips of reserpine-treated rabbits.

In conclusion, reserpine-induced supersensitivity has been demonstrated in the isolated atria of guinea-pigs to both the rate and tension responses. This was found to be selective for the β-adrenoceptor agonists isoprenaline and salbutamol. Whether this is due to an increased affinity or efficacy or receptor proliferation has yet to be resolved, however, the partial agonist salbutamol may offer an opportunity to examine these possibilities.

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