Pharmacological Properties of Presynaptic β -Adrenoceptors in Guinea-Pig Pulmonary Arteries

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Abstract—Pharmacological properties of the facilitatory presynaptic β -adrenoceptor mechanism were studied in superfused spiral preparations of guinea-pig pulmonary arteries preloaded with ^3H -norepinephrine. (–)-Isoproterenol (0.3 μM)-induced increases in total ^3H efflux per pulse evoked by transmural field stimulation (1, 5, 10 and 20 Hz, 10 V, 2 msec pulse width, 100 pulses and 30 min intervals) were neither dependent on impulse-frequencies nor selective at lower frequencies. Isoproterenol increased ^3H efflux at 5 Hz in a concentration-dependent manner (1 nM to 1 μM): pD_2 was 7.7. Salbutamol increased ^3H efflux in a similar manner to isoproterenol: pD_2 was 7.4. Prenalterol at 3 μM only slightly increased ^3H efflux. Tazolol (10 nM to 3 μM) produced no increases. Atenolol (3 μM) and practolol (3 μM) did not antagonize isoproterenol (0.3 μM)-induced increases in ^3H efflux. Butoxamine (3 μM) and H 35/25 (3 μM) did antagonize this parameter. (–)-Epinephrine (1 nM to 0.1 μM) decreased ^3H efflux at 5 Hz and concentration-dependently increased this parameter in the presence of 10 μM phentolamine. (–)-Norepinephrine (10 nM to 1 μM) concentration-dependently inhibited evoked ^3H efflux and did not increase the parameter in the presence of 10 μM phentolamine, 10 μM cocaine and 10 μM normetanephrine. Thus, there exist presynaptic β_2 -subtype receptors on noradrenergic nerve endings innervating guinea-pig pulmonary arteries.

The role of presynaptic α -adrenoceptors in the regulation of the release of the transmitter norepinephrine through a negative feedback mechanism has been well established (1–3), and it has been proposed that there is an additional facilitatory mechanism via presynaptic β -adrenoceptors (4–7). It has been considered that this presynaptic facilitatory mechanism is most sensitive at low impulse-frequencies and low biophase concentrations of norepinephrine leading to an increase in norepinephrine release (1). An alternative

hypothesis is that the facilitatory mechanism is mediated by circulating epinephrine of adrenal medullary origin rather than by the positive feedback by norepinephrine (8). Misu et al. (9) demonstrated that presynaptic β -adrenoceptors are present on the surface membrane of noradrenergic nerve endings innervating guinea-pig pulmonary arteries and that low doses of (–)-propranolol inhibit transmission via blockade of these receptors.

In the present experiments, pharmacological properties of these presynaptic β -adrenoceptors were studied in the guinea-pig pulmonary arteries preloaded with ^3H -norepinephrine to determine frequency-efflux relationships and dose-efflux relationships of relatively selective β_1 - and β_2 -adrenoceptor agonists and blocking activities of relatively selective β_1 - and β_2 -adrenoceptor antago-

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nists. Actions of norepinephrine and epinephrine were further studied to elucidate the endogenous role of the presynaptic β -adrenoceptor mechanism. A preliminary report has already appeared (10).

Materials and Methods

Male guinea-pigs weighing 200 to 250 g were sacrificed by a blow on the head, and main pulmonary arteries were excised. The spirally cut preparations were prepared as described previously (9) and were incubated at 37°C, pH 7.2 to 7.4, for 60 min in Krebs bicarbonate solution, bubbled with 95% O₂-5% CO₂, containing 0.1 μ M (-)-[7, 8-³H]-norepinephrine (5 μ Ci/ml, specific activity 38.6 Ci/mmol, The Radiochemical Center), 5.7 $\times$ 10⁻⁴ M ascorbic acid and 4.0 $\times$ 10⁻⁶ M EDTA. The composition of Krebs' solution was as follows (in mM): NaCl, 118.4; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.18; NaHCO₃, 25; KH₂PO₄, 1.2 and glucose, 11.1. After rinsing for 10 min with norepinephrine-free solution, the preparations were vertically mounted between a pair of platinum stimulation electrodes of 0.5 mm diameter and separated by 2 mm along the whole length of the strips (approximately 15 mm, weighing 5 to 6 mg) and were superfused with Krebs' solution at a constant flow rate of 1 ml/min utilizing a microtube pump (MP-3A, Tokyo Rikakikai). The resting tension was adjusted to 0.5 g. Transmural field stimulation with electrical rectangular pulses (1, 5, 10 or 20 Hz, 10 V, 2 msec pulse width, 100 pulses and usually 30 min intervals) was performed by use of an electrical stimulator (SEN 3201, Nihon Kohden). In all preparations, a stimulation with 1 Hz for 100 sec or with 5 Hz for 20 sec was applied only to "condition" the strips 60 min after the onset of superfusion and evoked ³H efflux was disregarded. A 30 min equilibration period was further allowed before superfusion of drug solution.

Superfusate was collected into a vial 3 times, before, during and after field stimulation, for 1 min in the cases with 5, 10 and 20 Hz or for 2 min with 1 Hz, respectively. ACS-II solution (Amersham/Searle) was added (6 ml/1 ml of the superfusate), and total ³H activities expressed as disintegrations per min were determined using a liquid

scintillation spectrometer (Packard 2660). Impulse-evoked increases in ³H efflux were calculated as the difference between basal efflux before stimulation and total efflux detected in the successive 2 samples during and after stimulation. Isotonic contractile responses to transmural field stimulation were monitored by an inkwriting oscillograph (Biophysigraph 180 Systems, San-ei).

Drugs used were cocaine hydrochloride (Takeda); phentolamine hydrochloride (Ciba-Geigy); normetanephrine hydrochloride, (-)-norepinephrine bitartrate, (-)-epinephrine bitartrate and (-)-isoproterenol hydrochloride (Sigma); salbutamol sulfate (Sankyo); tazolol hydrochloride (Syntex); prenalterol hydrochloride and ($\pm$)-erythro-4'-methyl- α -(1-isopropylaminoethyl) benzylalcohol hydrochloride (H 35/25) (A. B. Hässle); butoxamine hydrochloride (Burroughs-Wellcome); propranolol hydrochloride, atenolol hydrochloride and practolol hydrochloride (ICI). (-)-Norepinephrine and (-)-epinephrine were dissolved in 0.06 N HCl and the other drugs in saline. Preparations were usually exposed for 30 min to the superfusion medium containing drugs. Some experiments regarding (-)-epinephrine and (-)-norepinephrine were performed 30 min after the pretreatment with phentolamine or phentolamine, cocaine and normetanephrine.

The pD₂ values of some β -adrenoceptor agonists on increases in impulse-evoked ³H efflux were calculated by the methods described by Van Rossum (11). Student's *t*-test was used to evaluate data significance.

Results

Total ³H efflux by transmural field stimulation from spirally cut pulmonary arteries preloaded with ³H-norepinephrine: Resting ³H efflux abruptly and exponentially decreased after the beginning of superfusion with Krebs' solution (9), and that immediately before transmural field stimulation 90 min after superfusion was 684.3 $\pm$ 60.1 dpm/mg wet tissue (n=4). The absolute value of ³H efflux varied from preparation to preparation. Transmural field stimulation at 1 Hz for 100 sec released a total ³H of 818.1 $\pm$ 30.0 dpm/mg wet tissue/pulse. Thereafter, the amount of ³H evoked by stimulation with 1 Hz at

30 min intervals remained almost the same for at least 2 hr (9). As demonstrated in Fig. 1, there was an increase in impulse-evoked efflux/pulse, as the frequency was increased from 1 to 20 Hz.

Effects of β -adrenoceptor agonists on impulse-evoked ^3H efflux: (-)-Isoproterenol at 0.3 μM significantly increased ^3H efflux evoked by stimulation with 100 pulses at each frequency, respectively (Fig. 2). These increases, however, were neither dependent on impulse frequencies nor selective at lower frequencies.

Resting ^3H efflux and impulse-evoked ^3H efflux with 5 Hz were 318.7 ± 42.4 dpm/mg wet tissue and 1151.4 ± 392.0 dpm/mg wet tissue/pulse ($n=6$), respectively 90 min after the start of superfusion. Isoproterenol increased ^3H efflux evoked by impulses at 5 Hz in a concentration-dependent manner from 1 nM to 1 μM (Fig. 3): the calculated pD_2 value was 7.7. Salbutamol markedly increased evoked efflux in a similar manner to isoproterenol: pD_2 value was 7.4. On the other hand, 3 μM prenalterol only slightly increased evoked efflux. Tazolol, 0.1 to 3 μM ,

produced no increases in the parameter.

Effects of β -adrenoceptor antagonists on isoproterenol-induced increases in impulse-evoked ^3H efflux: Atenolol, 0.3 and 3 μM , and practolol, 1 and 3 μM , did not antagonize isoproterenol (0.3 μM)-induced increases in ^3H efflux evoked by 100 pulses at 5 Hz (Fig. 4). On the other hand, 3 μM butoxamine and 3 μM H 35/25 significantly antagonized this parameter.

Effects of (-)-epinephrine and (-)-norepinephrine on impulse-evoked ^3H efflux: Epinephrine, 1 to 100 nM, inhibited ^3H efflux evoked by 100 pulses at 5 Hz in untreated tissues (Table 1). This inhibition was not concentration-dependent. Pretreatment with 10 μM phentolamine markedly increased evoked efflux: a ratio of evoked efflux before and after the pretreatment was $267.5 \pm 25.5\%$, compared to the control ratio, $94.9 \pm 0.7\%$. In the presence of phentolamine, epinephrine concentration-dependently increased evoked efflux. On the other hand, 10 nM to 1 μM norepinephrine concentration-dependently inhibited evoked ^3H efflux. Pretreatment with 10 μM phentolamine under conditions of

Fig. 1. Frequency-efflux relationship of impulse-evoked ^3H efflux from spirally cut pulmonary arteries of guinea-pigs. Strips preloaded with ^3H -norepinephrine were superfused with Krebs' solution. Ordinate shows impulse-evoked ^3H efflux expressed as a % of control with 100 pulses at 1 Hz 90 min after superfusion. Thereafter, transmural field stimulation was performed every 30 min with 100 pulses at 5, 10 and then 20 Hz. Vertical bar shows S.E., parenthesis numbers of estimations. * $P < 0.05$, compared with the control.

Fig. 2. Frequency-independent facilitatory actions of 0.3 μM (-)-isoproterenol (hatched columns) on impulse-evoked ^3H efflux. Isoproterenol was superfused 90 min after setting up strips. Stimulation at a fixed frequency of 1, 5, 10 or 20 Hz was performed twice before (S_1) and 30 min after (S_2) the start of superfusion with isoproterenol. Abscisse shows ^3H efflux by S_2 expressed as a % of that by S_1 . Open columns show control, horizontal bars S.E., parentheses numbers of estimations. * $P < 0.01$, compared with 0 μM . Further details as in Fig. 1.

Fig. 3. Dose-efflux curves for isoproterenol ($\circ$), salbutamol ($\square$), prenalterol ($\triangle$) and Tazolol ($\times$) on facilitation of impulse-evoked ^3H efflux. Strips were superfused with each concentration of β -adrenoceptor agonists 90 min after setting up. Abscissa shows $-\log$ concentrations. Stimulation was performed twice at 5 Hz before (S_1) and 30 min after (S_2) drug superfusion. Ordinate shows ^3H efflux by S_2 expressed as a % of that by S_1 , which is further divided by S_2/S_1 efflux ratio in untreated strips demonstrated in Fig. 2. Each point represents the mean of 4 to 6 estimations. Further details as in Figs. 1 and 2.

Fig. 4. Antagonizing effects of β -adrenoceptor antagonists on isoproterenol ($0.3 \mu\text{M}$)-induced increases in impulse-evoked ^3H efflux. Stimulation was done at 5 Hz. β -Adrenoceptor antagonists were superfused simultaneously with isoproterenol. Further details as in Figs. 1 and 2.

inhibition of uptake₁ and uptake₂ by $10 \mu\text{M}$ cocaine and $10 \mu\text{M}$ normetanephrine (12) increased the ratio of evoked efflux to $243.8 \pm 11.6\%$. It was difficult for phentolamine to

antagonize the inhibition induced by lower concentrations of norepinephrine under these conditions, but the inhibition elicited by $1 \mu\text{M}$ norepinephrine was almost completely abolished. However, no increases in evoked ^3H efflux over the control level were seen. The norepinephrine ($1 \mu\text{M}$)-induced inhibition, abolished in the presence of phentolamine, cocaine and normetanephrine, was significantly restored by the additional superfusion with $1 \mu\text{M}$ (-)-propranolol.

Resting ^3H efflux: It was slightly increased by epinephrine. When a ratio of resting ^3H efflux immediately before S_3 and S_2 periods of field stimulation in experiments shown in Table 1 was calculated, the values of the ratio 30 min after superfusion with 1, 10 and 100 nM epinephrine were $79.8 \pm 2.8\%$, $83.7 \pm 3.8\%$ and $122.7 \pm 11.9\%$, respectively, compared to the control ratio, $87.9 \pm 3.0\%$. Increases in the parameter were not seen in the presence of phentolamine. On the other hand, norepinephrine concentration-dependently increased resting ^3H efflux: the values of the ratio after 10, 100 nM and $1 \mu\text{M}$ norepinephrine were $98.9 \pm 3.0\%$, $130.3 \pm 4.5\%$ and $491.6 \pm 61.8\%$, respectively. In the presence of phentolamine, cocaine and normetanephrine, $1 \mu\text{M}$ norepi-

Table 1. Effects of epinephrine and norepinephrine on impulse-evoked ^3H efflux from spiral strips of guinea-pig pulmonary arteries

Pretreatments	Agonists	Doses (nM)	N	Evoked ^3H efflux (% S_3/S_2)
None	Epinephrine	Control	6	97.1 $\pm$ 1.6
		1	4	77.2 $\pm$ 5.4**
		10	4	73.7 $\pm$ 9.8*
		100	4	75.4 $\pm$ 5.0**
Phentolamine	Epinephrine	Control	8	88.6 $\pm$ 1.5
		1	4	91.8 $\pm$ 2.3
		10	4	103.0 $\pm$ 2.5**
		100	4	131.6 $\pm$ 7.1**
None	Norepinephrine	Control	6	97.1 $\pm$ 1.6
		10	9	78.3 $\pm$ 4.2**
		100	8	76.3 $\pm$ 6.3**
		1000	4	49.7 $\pm$ 3.4**
Phentolamine, cocaine and normetanephrine	Norepinephrine	Control	4	89.1 $\pm$ 2.3
		10	6	72.9 $\pm$ 4.3**
		100	4	81.6 $\pm$ 5.7
		1000	4	85.8 $\pm$ 2.8
Phentolamine, cocaine, normetanephrine and propranolol	Norepinephrine	1000	4	62.4 $\pm$ 3.3†

Stimulation was performed three times (S_1 to S_3 periods) with a frequency of 5 Hz for 20 sec at 30 min intervals. Evoked ^3H efflux by S_2 and S_3 is expressed as a % of that by S_1 and S_2 , respectively. Epinephrine or norepinephrine was superfused after S_2 . Pretreatment with 10 μM phentolamine or with 10 μM phentolamine, 10 μM cocaine and 10 μM normetanephrine was performed after S_1 . (-)-Propranolol, 1 μM , was further superfused simultaneously with norepinephrine after S_2 in the presence of the 3 blockers. Data shown are means $\pm$ S.E. of N experiments. * $P < 0.05$, ** $P < 0.01$, compared to the control; † $P < 0.01$, compared to 1 μM in the presence of the 3 blockers.

nephrine alone increased this ratio (106.0 $\pm$ 5.1%, compared to the control, 73.4 $\pm$ 6.3%). These epinephrine- and norepinephrine-elicited increases in resting ^3H efflux are probably due to blockade of the uptake mechanism of ^3H norepinephrine (12).

The application of the other β -adrenoceptor agonists and the combined application of β -adrenoceptor antagonists and isoproterenol produced no modifications of resting ^3H efflux at the range of concentrations studied.

Discussion

In spirally cut strips of guinea-pig pulmonary arteries preloaded with ^3H -norepinephrine, the amount of total ^3H efflux per pulse evoked by field stimulation increased as the frequency was increased (1 to 20 Hz). This result is in accordance with that in many

sympathetically innervated tissues such as cat spleen (13), uterine artery (14) and vas deferens (15) of guinea-pigs and rabbit portal vein and vas deferens (16).

Isoproterenol increased impulse-evoked ^3H efflux. However, the increases were independent of impulse frequencies, and low frequency-selective increases such as those demonstrated in the human oviduct (17), the heart in vivo (18) and the saphenous vein in vitro (19) of dogs were not seen. Furthermore, isoproterenol increased evoked ^3H efflux, in a concentration-dependent manner at wide ranges from low to high concentrations (1 nM to 1 μM): low concentration-selective increases were not seen, as those demonstrated in the human oviduct (17), the guinea-pig atria (5) and the perfused cat spleen (1). The present results show that the

facilitatory presynaptic β -adrenoceptor mechanism is not always operative only at low frequencies of nerve stimulation and at low biophase concentrations of the transmitter norepinephrine and other adrenergic agonists. Our data appear not to be consistent with the hypothesis that a positive feedback mechanism for the transmitter release would be initiated by low biophase concentrations of norepinephrine (1).

Salbutamol, a relatively selective β_2 -adrenoceptor agonist, produced a dose-efflux curve almost similar to that seen with isoproterenol. On the other hand, prenalterol (20) and tazolol (21), relatively selective β_1 -adrenoceptor agonists, produced only slight or no increases in the impulse-evoked ^3H efflux, respectively, although it should be taken into account that these partial agonists have β -adrenoceptor blocking properties (22, 33). Atenolol (24) and practolol (25), relatively selective β_1 -adrenoceptor antagonists, did not antagonize isoproterenol-induced increases in the evoked ^3H efflux within the dose ranges and at the frequency used (5 Hz), whereas butoxamine (26) and H 35/25 (25, 27), relatively selective β_2 -adrenoceptor antagonists, antagonized this parameter. Furthermore, we confirmed that presynaptic β -adrenoceptors in guinea-pig pulmonary arteries have characteristics similar to those of postsynaptic classical β -adrenoceptors (7, 28). All of these results clearly demonstrate that there exist presynaptic β_2 -subtype adrenoceptors on the surface membrane of adrenergic nerve endings innervating guinea-pig pulmonary arteries. Similar results were obtained in human omental arteries and veins (8) and in the portal vein of normotensive rats (29) and of spontaneously hypertensive rats (30). These results stand in contrast to the findings obtained in the perfused blood vessels of cat hindlimb preparations: Dahlöf et al. (31) suggested presynaptic β_1 -subtype adrenoceptors on the basis of the inhibitory action of metoprolol on vasopressor responses to lumbar sympathetic nerve stimulation and on norepinephrine release. These results demonstrate the probability that the facilitatory presynaptic β -adrenoceptor mechanism is mediated via different subtypes in different tissues and

species. Furthermore, the coexistence of presynaptic β_1 - and β_2 -adrenoceptors on catecholaminergic nerve endings is demonstrated in the posterior hypothalamus of cats (32) and in the hypothalamic slices of rats (33, 34).

Epinephrine, an α -, β_1 - and β_2 -agonist, decreased evoked ^3H efflux and the decreases were not concentration-dependent in the absence of phentolamine, whereas the agonist readily increased in a concentration-dependent manner this parameter in the presence of phentolamine. Phentolamine unmasked a facilitatory action of epinephrine. The epinephrine-induced concentration-independent decreases in the absence of phentolamine may be due to a balance between biphasic negative and positive feedback mechanisms on evoked efflux of the transmitter. On the other hand, norepinephrine, an α - and β_1 -agonist with a slight β_2 -activity, concentration-dependently inhibited evoked ^3H efflux in the absence of phentolamine, cocaine and normetanephrine; and no increases in this parameter over the control level were seen even by the highest concentration of norepinephrine ($1\text{ }\mu\text{M}$) accompanied with increases in resting ^3H efflux in the presence of phentolamine, cocaine and normetanephrine. The inhibition elicited by the highest concentration of norepinephrine appears to be "fully" antagonized by phentolamine. However, this antagonism probably involves the " β_2 -agonistic facilitatory activity" of norepinephrine for the transmitter release, since the norepinephrine-induced inhibition of ^3H release, abolished in the presence of phentolamine, cocaine and normetanephrine, was restored again by the additional presence of (-)-propranolol. Similar propranolol-elicited "reversal" of the effects of norepinephrine and epinephrine is also found in the presence of phenoxybenzamine in rat portal vein (30). Phentolamine alone markedly increased evoked ^3H efflux, which probably demonstrates that phentolamine antagonizes a negative feedback action of neuronally released norepinephrine on the transmitter release (1–3). However, it was difficult for phentolamine ($10\text{ }\mu\text{M}$) to antagonize the inhibition of evoked ^3H efflux induced by lower concentrations of

norepinephrine. The reason for this incomplete antagonism against exogenously applied norepinephrine is not clear at present.

Concerning the role of endogenous norepinephrine and epinephrine for the presynaptic β -adrenoceptors, (–)-propranolol alone inhibited contractile responses to sympathetic nerve stimulation, whereas the (+)-isomer produced no inhibition of transmission and a dissociation between inhibitory actions of the (–)- and (+)-isomers on evoked ^3H efflux was seen in the presence of phentolamine in isolated guinea-pig pulmonary arteries (9). These facts and results obtained in the present characterization experiments suggest that neuronally released norepinephrine can increase its own release via the facilitation of β_2 -adrenoceptors. This idea supports to some extent the Langer hypothesis (1) that neuronally released norepinephrine is concerned in the enhancement of the transmitter release. In fact, postsynaptic vascular β_2 -adrenoceptors can be stimulated by neuronally released norepinephrine, and resulting vasodilatation is demonstrated in the feline pulmonary vascular bed (35) and in the facial vein of the rabbits (36). However, the present results regarding exogenously applied epinephrine and norepinephrine suggest that peripheral presynaptic facilitatory β -adrenoceptors are readily activated by circulating epinephrine (8) or epinephrine taken-up and released as a cotransmitter (19, 37, 38) rather than by norepinephrine itself released from noradrenergic nerve endings (6, 9). This idea is also indirectly supported by our own data that in rat hypothalamic slices, inhibitory actions of (–)-propranolol on impulse-evoked release of endogenous norepinephrine and dopamine were abolished when endogenous contents of epinephrine were markedly reduced by the pretreatment with 2, 3-dichloro- α -methylbenzylamine, an inhibitor of phenylethanolamine N-methyltransferase (34 and H. Ueda et al., unpublished data).

In conclusion, there exist presynaptic β_2 -subtype adrenoceptors on the adrenergic nerve endings innervating the guinea-pig pulmonary arteries.

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