DESENSITIZATION OF GUINEA PIG TRACHEAL MUSCLE PREPARATION TO BETA-ADRENERGIC STIMULANTS BY A PRECEDING EXPOSURE TO A HIGH DOSE OF CATECHOLAMINES

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Abstract—When a given concentration of a catecholamine was applied to guinea pig tracheal preparation contracted by 20 μM histamine or by 30 mM-K+ Tyrode's solution, constant relaxations were observed if the relaxation was submaximal. When a high concentration of catecholamine, 200 times the ED50, was once applied, subsequent responses to beta-stimulants (ED80) was reduced by about 30-40%, in spite of repeated washings. The response was gradually recovered in 2 hr. Thus 45 μM epinephrine and 1 μM isoproterenol could cause desensitization to 0.65 μM epinephrine and 0.03 μM isoproterenol, respectively. Epinephrine and isoproterenol could cause desensitization to isoprophenamine, a non-catechol beta-stimulant. Epinephrine did not affect the response to cyclic AMP, dibutyryl cyclic AMP, aminophylline and prostaglandin E2. This desensitization was not affected by phenolamine, normetanephrine nor by Ca²⁺ deprivation from the bathing solution. The mechanisms of the desensitization may relate to some steps between the receptor-drug interaction and cyclic AMP accumulation in the process of tracheal muscle relaxation induced by beta-stimulants.

Adrenergic beta-stimulants are widely used for the treatment of broncheal asthma. This type of drug has, however, serious adverse effects: 1) palpitation of the heart, 2) tolerance development in the bronchodilating effect and, in addition, occasional induction of bronchoconstriction, 3) sudden death of patients strongly dependent on these drugs. Introduction of a new type of beta-stimulant, so-called 'beta,-adrenoceptor-specific stimulants', e.g., salbutanol, almost eliminated the side effects on the heart. The latter two adverse effects have yet to be overcome. Although some possible mechanisms have been proposed for these adverse effects, there is no generally accepted explanation. It is quite likely, however, that a desensitization of beta-adrenoceptors which is produced by repeated topical or systemic administrations of the beta-stimulant plays an important role.

Watanabe et al. (1) have shown that the effect of a non-catechol beta-adrenergic bronchodilator, isoprophenamine, is reduced after a treatment with a high concentration of epinephrine using guinea pig tracheal muscle preparation in vitro. Subsequent intensive studies have revealed that not only the effects of isoprophenamine but also those of isoproterenol, epinephrine and norepinephrine are reduced by a preceding application of a high

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concentration of any beta-adrenoceptor stimulant. Paton's rate theory (2) explains some of desensitization phenomena, but seems unapplicable to the beta-adrenoceptor-adenylate cyclase system.

The present paper describes the desensitization phenomena and the results of the investigation of the mechanisms of these phenomena.

MATERIALS AND METHODS

Male guinea pigs weighing 350 to 450 g were sacrificed by a blow on the head, the trachea was excised and connective tissues were removed. Tracheal strip chain preparations were then made following the method of Takagi et al. (3). The strip was suspended in 6 ml-organ bath filled with Tyrode's solution and aerated with 5% CO₂ and 95% O₂ at 35-36°C. Drug-induced responses were recorded isotonically with a magnification of 7 times. The tension applied on the strip was 0.3 g.

A typical experimental procedure is shown in Fig. 1. The strip was contracted by histamine (20 μM) and, when the response reached a maximum, medium concentration of a dilator was applied. The drugs were washed out 15 min later. Seventy min later, a high concentration of dilator was applied for 15 min to the strip contracted by histamine. Thereafter, the time course of the response to medium concentration of the dilator was examined. Interval of applications of the dilator was kept at 70 min. Another preparation from the same animal was used as a control. When dibutyryl cyclic AMP was used as a dilator, the preparation was exposed for 20 min, as the effect developed slowly. Thirty-mM-K⁺-Tyrode's solution, in which NaCl was reduced to adjust the osmotic pressure, was also used as spasmodogen in place of histamine.

![Fig. 1. Illustration of the sequence of drug application for the assessment of desensitization caused by a high concentration of relaxants. ⬤: Histamine, 20 μM; ○: medium concentration of relaxant (ED80); ◊: conditioning high concentration of relaxant (200 × ED50).](image)

The results are indicated as percent of the initial relaxing response with standard errors of the mean.

The following drugs were used. Abbreviations used in this paper are also indicated. dl-Epinephrine hydrochloride (Epi) (Sankyo Co.), histamine dihydrochloride, aminophylline and disodium glycoletherdiaminetetraacetate (GEDTA) (Wako-Jun-Yaku Co.), 1-nor-epinephrine hydrochloride (NE) and murexide (Tokyo-Kasei Co.), 1-isoproterenol hydrochloride (Iso) (Eizai Co.)*, phentolamine methanesulfonate (CIBA Ltd.), propranolol hydrochloride (Sumitomo-Kagaku Co.)*, MJ 1999 hydrochloride (Mead-Johnson Co.)*, dl-normetanephrine hydrochloride (Sigma), adenosine 3',5'-cyclic monophosphate (cyclic
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AMP) (Wakamoto-Seiyaku Co.)*, dibutyril adenosine 3',5'-cyclic monophosphate (DB cyclic AMP) (Kyowa-Hakko-Kogyo Co.)*, prostaglandin E₁ (PGE₁) (Ono-Seiyaku Co.)*. Asterisk indicates that these drugs were gifts from the makers to whom we are grateful.

RESULTS

Desensitization to isoprophenanfine caused by catecholamines

Dose-response curves for relaxing effect of Epi, Iso and NE on histamine-induced spasm of guinea pig tracheal preparation are shown in Fig. 2. From these curves ED50 values were determined.

The influence of a high concentration (ED50 x 200) of Epi (45 μM) and Iso (1 μM) on the response to medium concentration (ED80) of Ipp (10 μM) was studied. ED50 x 200 corresponds to about 3 x ED100. It was found that the Epi- and Iso-pretreatment reduced the sensitivity of the preparation to Ipp to 24.5 and 52.5% of initial response, respectively (Table 1). Extent of the histamine-induced contractions of each preparation remained almost constant over the several serial applications. In some experiments in which relaxing effect on the intrinsic tone of the preparation was observed using no spasmogen, desensitization of the same extent could be observed.

Desensitization to Epi, Igg and NE caused by themselves

High concentration of Iso or Epi caused a desensitization also to medium concentration of Iso or Epi, respectively. The desensitizations caused by both catecholamines were quite similar in degree and in the time course of recovery (Fig. 3). Similar desensitization was

Table 1. Desensitization to Ipp (10 μM) caused by a high dose of Epi (45 μM) or Iso (1 μM) in the guinea pig tracheal preparation

|        | Relaxation (percent initial) | Significance p< |
|--------|-----------------------------|-----------------|
|        | Treated                     | Control         |
| Epi    |                             |                 |
| 70     | 24.5 ± 2.4 (7)              | 94.5 ± 3.6 (8)  | 0.001 |
| 140    | 50.9 ± 4.4 (5)              | 77.9 ± 3.5 (6)  | 0.01  |
| Iso    |                             |                 |
| 70     | 52.5 ± 4.3 (6)              | 106.2 ± 7.5 (6) | 0.001 |

1: Time after the conditioning catecholamine application.
Figures in parentheses indicate the number of experiments. In control experiments, 0.65 μM Epi or 0.03 μM ISO was applied in place of high doses.
Fig. 3. Desensitization of the guinea pig tracheal smooth muscle to Epi and Iso caused by high concentrations of Epi and Iso, respectively. (a) Epi, (b) Iso. Difference as compared with control was statistically significant at 5% (*) and 1% (**) level. Each point on the lines represents the relative height of relaxation (initial height = 100) caused by 0.65 μM Epi (a) or 0.03 μM Iso (b). •: Controls; ○: desensitized preparations. At 70 min conditioning dose of Epi (45 μM) (a) or Iso (1 μM) (b) was applied. Number of experiments 7-9.

Table 2. Desensitization to catecholamines caused by a high dose of Epi (45 μM) in the guinea pig tracheal preparation

| Time (min) | Relaxation (percent initial) | Significance |
|------------|-----------------------------|--------------|
|            | Treated                     | Control      | p*   |
| Epi 0.65 μM|                             |              |      |
| 60         | 62.1 ± 4.1 (5)              | 100.3 ± 5.6 (5) | 0.001 |
| 70         | 72.3 ± 8.8 (7)              | 100.3 ± 1.7 (7) | 0.02  |
| 80         | 83.3 ± 5.3 (6)              | 99.3 ± 1.8 (6) | 0.05  |
| NE 10 μM   |                             |              |      |
| 60         | 47.8 ± 9.1 (5)              | 72.5 ± 5.2 (5) | 0.05  |
| Iso 0.03 μM|                             |              |      |
| 60         | 61.3 ± 11.9 (5)             | 121.3 ± 8.1 (5) | 0.01  |

*: Time after the conditioning Epi application. Figures in parentheses indicate the number of experiments. In control experiments, 0.65 μM Epi was applied in place of a high dose of Epi. Thirty mM-K⁺-Tyrode's solution was used as spasmogen.

also observed when 30 mM-K⁺-Tyrode's solution was used as spasmogen instead of histamine. Table 2 shows the desensitizations to Epi, Iso and NE caused by the high concentration of Epi using K⁺ as spasmogen.

Possible contribution of alpha-adrenoceptor to the desensitization was examined, although catecholamines did not induce any contractile response of the preparation in the presence of beta-adrenoceptor blocker, propranolol (10 μM) or MJ 199 (10 or 100 μM). As indicated in Fig. 4, presence of 2.7 μM phenolamine, an alpha-adrenoceptor blocker, in the medium throughout the experiment did not affect the Epi-induced desensitization to Epi. This dose of phenolamine is known to sufficiently block the medium alpha-
Influence of phentolamine (2.7 μM) on the Epi-induced desensitization to Epi in the guinea pig tracheal muscle preparation. 30 mM-K⁺-Tyrode's solution was used as spasmogen. •: Controls; ○: in the presence of phentolamine. Points at 70 min represent the response to 45 μM Epi. Other points represent the relative height of relaxation (initial height = 100) caused by 0.65 μM Epi. Number of experiments was 5 or 6.

Influence of extraneuronal uptake inhibition on the desensitization

dl-Normetanephrine is known to inhibit the extraneuronal catecholamine-uptake (4). Since there is the possibility that an increase in the concentration of catecholamines in smooth muscle cells might reduce the catecholamine-induced relaxation, the influence of extraneuronal uptake inhibitor on the desensitization was examined. The results shown in Fig. 5 indicate that the uptake inhibition did not affect the desensitization.

Aminophylline, cyclic AMP and DB cyclic AMP

Since it is suggested that the relaxing effect of beta-adrenoceptor stimulants is mediated by the intracellular increase in cyclic AMP, the possibility of implication of this process in the mechanism of the desensitization was explored by examining the effect of cyclic AMP, DB cyclic AMP, and aminophylline which is said to relax smooth muscle through phosphodiesterase inhibition and resulting cyclic AMP accumulation (5). In Fig. 6 relaxing effect of these drugs in the presence of histamine (20 μM) is shown. ED80 of cyclic AMP was 80 times that of DB cyclic AMP, but the onset and development of the effect were much slower in the latter than in the former.

The influence of a high concentration of Epi on the effect of these drugs was then ex-
Fig. 6. Cumulative dose-response curve for relaxing effect of aminophylline, cyclic AMP and DB cyclic AMP in the guinea pig tracheal muscle preparation. Histamine, 20 μM, was used as spasmogen. Number of experiments 8–9.

Fig. 7. Influence of high concentration of Epi on the response to aminophylline in the guinea pig tracheal preparation. Each point represents the relative height of relaxation (initial height = 100) caused by 0.2 mM aminophylline. At 70 min 45 μM (○) or 0.65 μM (●) Epi was applied. Number of experiments 7–9.

| Conc. (mM) | t (min) | Relaxation (percent initial) | Significance |
|-----------|---------|-----------------------------|-------------|
|           |         | Treated                     | Control     |             |
| Cyclic AMP| 30      | 103.5 ± 1.9 (9)             | 100.4 ± 1.1 (9) | N.S.        |
| DB cyclic AMP| 1       | 95.3 ± 1.1 (5)             | 85.3 ± 9.4 (6) | N.S.        |

1: Time after the conditioning Epi application. Figures in parentheses indicate the number of experiments. In control experiments, 0.65 μM Epi was applied.

Table 3. Lack of desensitization to cyclic AMP and DB cyclic AMP following a high dose of Epi (45 μM) in guinea pig tracheal preparation

amined. No significant change was detected in the effect of the drugs (Table 3 and Fig. 7). In turn, a high concentration of aminophylline (15 mM) did not cause any desensitization to medium concentration of aminophylline (0.2 mM) (Fig. 8).

Influence of a high concentration of Epi on the sensitivity to PGE₁

It is very unlikely that the relaxation of tracheal muscle induced by PGE₁ is mediated by beta-adrenoceptors, although change in cyclic AMP level might be implicated in the mechanisms (6, 7). As in Table 4, sensitivity to PGE₁ was unaffected by an application of the high concentration of Epi, whether the spasmogen was histamine or K⁺.

Influence of Ca²⁺-chelating agent

In order to obtain some insight into the possibility of implication of Ca²⁺ in the desensitization phenomenon, the high concentration of Epi was applied in the presence of 2 mM
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FIG. 8. Influence of single application of a high concentration of aminophylline to the response to aminophylline of the guinea pig tracheal preparation. Each point represents the relative height of relaxation (initial height = 100) caused by 0.2 mM aminophylline. At 70 min, 15 mM aminophylline was applied (○). •: Control. Number of experiments was 8.

FIG. 9. Influence of 2 mM GEDTA on the desensitization to Epi caused by Epi. Each point represents response to 0.65 µM Epi. •: Conditioned by GEDTA and 0.65 µM Epi; ○: conditioned by GEDTA and 45 µM Epi. Spasmogen: 30 mM-K⁺-Tyrode's solution. **: Significantly different from control (p<0.01). Number of experiments was 6.

Table 4. Lack of desensitization to PGE₁ following a high dose of Epi (45 µM) in the guinea pig tracheal preparation

| PGE₁ (µM) | t (min) | Relaxation (percent initial) | Significance |
|-----------|---------|----------------------------|-------------|
|           |         | Treated                     | Control     |
| Histamine 20 µM | 2.8    | 111.3 ± 13.1 (6) | 97.0 ± 15.2 (6) | N.S. |
| 30 mM-K⁺-Tyrode's soln. | 0.85 | 103.9 ± 18.5 (7) | 91.0 ± 11.4 (7) | N.S. |

1: Time after the conditioning Epi application. Figures in parentheses indicate the number of experiments. In control experiments, 0.65 µM Epi was applied in place of 45 µM Epi.

GEDTA, sufficient to chelate almost all Ca²⁺ in the medium. The latter was applied 2 min before the addition of the former. Under these conditions significant desensitization was still produced (p<0.01) as shown in Fig. 9.

In another experiment, a tracheal preparation was contracted by 30 mM K⁺ in Ca²⁺-free Tyrode's solution. Into this medium GEDTA (0.2 mM) was added, and the preparation was allowed to stand for 20 min. It was then exposed to the high concentration of Epi in this medium for 15 min as usual. In this preparation, the desensitization to medium concentration of Epi was observed when tested after 60 min (61 % of initial). The preparation was almost fully relaxed by the GEDTA, and very little relaxation occurred with the addition of the high concentration of Epi.
DISCUSSION

In the guinea pig tracheal muscle preparation, a supramaximal dose of beta-adrenoceptor stimulants caused a reversible desensitization to lower doses, without affecting the sensitivity to non-adrenergic bronchodilators. The desensitization caused by two catecholamines, Epi and Iso was of a similar extent, when extrapolated equi-effective concentrations (200 x ED50) were used as the supramaximal dose. This fact suggests that the desensitization is induced by a reversible change in the process(es) of development of the adrenergic beta-effect.

In the process, main steps which can be affected by the desensitizing procedure may be as follows: access of beta-adrenoceptor stimulant to the receptor sites, binding of the stimulant with the receptor, activation of receptor-adenylate cyclase complex, and decrease in intracellular Ca²⁺ concentration as influenced by cyclic AMP.

Iso is neither taken up by neurons (uptake.), nor metabolized by monoamine oxidase, and Ipp is not metabolized by catechol O-methyl transferase. The facts that a high concentration of Epi caused desensitization to Ipp and Iso, and that a high concentration of Iso also caused desensitization to Iso should exclude the possibility of the implication of neuronal uptake mechanism and of these catecholamine metabolizing enzymes in the desensitization mechanisms.

Normetanephrine, an inhibitor of extraneuronal catecholamine uptake, did not affect the desensitization. This fact also suggests that catecholamine metabolites remaining after washing do not inhibit the catecholamine binding to the receptor.

Epi did not cause desensitization to cyclic AMP, DB cyclic AMP, aminophylline and PGE₁. Considering the currently accepted mechanism of action for aminophylline (5) that this compound causes relaxation of smooth muscle by cyclic AMP accumulation via phosphodiesterase inhibition, it seems likely that there is no difference in the basal cyclic AMP production between the control and the preparation washed for 55 min after the 15 min-treatment with high concentration of Epi. It is said that cyclic AMP scarcely enters the cell, and that there is no substantial evidence that cyclic AMP applied in vitro exerts its action intracellularly. The most possible extracellular mechanism of action is binding of Ca²⁺ in the medium. This possibility was examined using murexide as [Ca²⁺] indicator (8), and was ruled out, because no detectable change in differential optical density (O.D.₅₄₀nm - O.D.₅₀₇nm) of 50 µM murexide was produced by 40 mM cyclic AMP-Na in the presence of 0.4 mM CaCl₂ (pH 6.8). Taken together it is not unreasonable to conclude that the desensitization is not caused by influence on the mechanism of cyclic AMP mediation of muscle relaxation.

As regards possible involvement of influence on Ca²⁺ movement, the results of the study using GEDTA indicate that the desensitization was still observed even when the high concentration of Epi was applied under circumstances which cause strong deficiency of intracellular Ca²⁺ necessary for muscle contraction. Thus the possibility of contribution of intracellular Ca²⁺ to the desensitization mechanism, e.g., saturation of intracellular Ca²⁺-binding sites etc., is very unlikely.

Fleish and Titus (9) found Iso-desensitization and reversal of the effect of Iso in rat
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aorta and that these changes were prevented by phentolamine, BOL, aminophylline, tetracaine, papaverine etc., and in the rat trachea, Iso, 15 μM, given every 15 min caused tachyphylaxis which could be prevented by tetracaine. In the application schedule, it was quite difficult to maintain the guinea pig tracheal preparation at a constant resting tone throughout an experiment and consequently to estimate the effect of catecholamines. As regards the desensitization described in this paper, no drug which prevents this phenomenon has been found.

Schumann et al. (10) have indicated that the decrease in pH of bath fluid reduces the affinity to Iso in the atrium and the ileum, but that in the trachea only a slight desensitization is seen. They attributed this to a low metabolic rate of the tracheal smooth muscle and proposed that the agonist-beta-adrenoceptor reaction depends on the intracellular pH which was changed by the metabolic state of the muscle. There seems to be a possibility that the desensitization described in the present paper may be caused by a long-lasting decrease in the intracellular pH due to a metabolic activation caused by a 15 min-contact with a high concentration of beta-adrenoceptor stimulants.

In conclusion, desensitization induced by a high concentration of beta-adrenoceptor stimulants is likely to be due to the reversible insufficiency of beta-adrenoceptor-adenylate cyclase complex function. As one of the possible causes, a change in intracellular pH should be considered.

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