INTERACTION BETWEEN ASPIRIN AND PROSTAGLANDINS IN THE ISOLATED GUINEA-PIG TRACHEAL MUSCLE

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Abstract—The effects of PGE₂ and PGF₂α on the tonus of isolated guinea-pig tracheal chain were investigated and compared with those of histamine and acetylcholine. PGE₂ reduced tonus in normal resting state, but elevated tracheal tonus reduced by aspirin. Such PGE₂-induced contractions did not exceed the initial resting tonus, and the magnitude and duration of the contractions progressively diminished with increase of PGE₂ concentrations. Aspirin produced neither relaxation nor contraction in the presence of a low dose of PGE₂. Unlike PGE₂, PGF₂α produced a dose-related contraction in the normal tracheal chain, and the contractile response to PGF₂α was markedly potentiated by aspirin. In the presence of PGF₂α, aspirin no longer produced tracheal relaxation but produced a dose-related contraction. The contractile effect of histamine but not of acetylcholine was also potentiated by aspirin, but there was a slight difference between PGF₂α and histamine in that the potentiation of action of PGF₂α by aspirin was more easily diminished by PGE₂. These results suggest that PGE₂ plays an important role in the maintenance of the resting tonus of the isolated guinea-pig tracheal chain, and in large doses it also acts as a tracheal relaxant and attenuates the tracheal responses to PGF₂α and histamine.

Aspirin-like drugs have been shown to inhibit prostaglandin (PG) biosynthesis in some 30 different systems (1, 2). Our previous report (3) showed that relatively low doses of aspirin-like drugs reduced dose-dependently the resting tonus of the isolated guinea-pig tracheal chain, and that there was a highly significant correlation between the degree of relaxation of the tracheal chain and the inhibitory effect on the contraction of the isolated rat stomach induced by arachidonic acid, a precursor of PGs. The antagonistic effect of aspirin-like drugs against arachidonic acid was considered to be due to inhibition of PG biosynthesis since the drugs had no direct antagonistic effect against PGs in the rat stomach fundus strips (3). The results suggest that the inhibition of PG biosynthesis may be responsible for the relaxation of the isolated guinea-pig tracheal chain.

It is well known that PGs have a broad-spectrum of pharmacological actions and exert different qualitative and quantitative activities. The bronchial and tracheal smooth muscles are generally found to be relaxed by treatment with PGEs, while PGFs produce contraction of the respiratory smooth muscles (4, 5). Therefore, the suggestion that the tracheal relaxation induced by aspirin-like drugs may be due to inhibition of PG biosynthesis leads to a possible explanation that aspirin-like drugs selectively inhibit biosynthesis of PGFs. However, contradictory evidence may be seen in the fact that bronchial tissue contains
predominantly PGEs and only small amounts of PGFs (6, 7). In addition, the fact that some asthmatic patients react severely to aspirin-like drugs also suggests that the drugs inhibit biosynthesis of PGEs, tracheal muscle relaxants.

To clarify these contradictions, we examined the effects of PGE₂, PGF₂α, histamine and acetylcholine on the isolated guinea-pig tracheal muscle whose endogenous PGs were depleted by aspirin, and a comparison was made with findings in the normal tracheal muscle.

MATERIALS AND METHODS

Male guinea pigs weighing 500 to 800 g were stunned and exsanguinated. Immediately thereafter, the trachea was removed from the animal and cut into small rings approximately 2 mm in width. Six tracheal rings were connected with a fine thread to make a chain. The chain was suspended in a 25-ml organ bath containing Tyrode's solution saturated with a gas mixture of 95% oxygen and 5% carbon dioxide. The temperature of the bath fluid was maintained at 37°C. The tracheal chain was connected to a force-displacement transducer under an initial tension of 0.6 g to 0.7 g, and the tonus was recorded isometrically on a polygraph. After suspension in an organ bath, the tonus of the tracheal chain began to decrease and reached the base 20 to 40 minutes later. The relaxation lasted 15 to 40 minutes, and then the preparation began to contract slowly, and 30 to 60 minutes later reached a steady state of 0.5 to 0.6 g tension.

The drugs used were as follows: aspirin (Hoei), isoproterenol hydrochloride (Sigma), histamine dihydrochloride (Nakairai), acetylcholine chloride (Daiichi), prostaglandin E₂ and F₂α (Sigma). Hereafter, prostaglandin E₂ and F₂α are referred to PGE₂ and PGF₂α, respectively.

RESULTS

Effect of PGE₂ on the tracheal tonus after pretreatment with aspirin: Although PGE₂ produced a dose-related reduction in the resting tonus of the isolated guinea-pig tracheal chain, it caused only contraction of the preparation of which the tonus had been almost completely reduced by aspirin of 1.0 x 10⁻⁵ to 1.6 x 10⁻⁴ g/ml (Fig. 1, 2). Even in a concentration as low as 6.4 x 10⁻¹⁰ g/ml which had no effect on the resting tonus, PGE₂ produced slight but persistent contraction. PGE₂ 2.5 x 10⁻⁹ g/ml restored the tracheal tonus reduced by aspirin to its initial resting tonus, and maintained it steadily at that level. The contraction produced by PGE₂ hardly exceeded the resting tonus even in large concentrations. The effects of PGE₂ at all concentrations from 2.5 x 10⁻⁹ to 4.0 x 10⁻⁵ g/ml were approximately the same. Moreover, the intensity and duration of the contractions were progressively reduced as the concentrations of PGE₂ were increased from 4.0 x 10⁻⁸ to 6.4 x 10⁻⁷ g/ml which produced clear-cut relaxation in the normal state (Fig. 2C).

Effect of PGF₂α on the tracheal tonus after pretreatment with aspirin: PGF₂α increased the tracheal tonus dose-dependently in concentrations of over 4.0 x 10⁻⁸ g/ml. The contraction peaked rapidly and then persisted steadily. The resting tonus of 0.5 to 0.6 g was increased to about 1.3 g by a concentration of PGF₂α 4.0 x 10⁻⁵ g/ml (Figs. 3, 4).
FIG. 1. Effect of PGE₂ on the isolated guinea-pig tracheal chain after treatment with aspirin. PGE₂ was added to the organ bath after the resting tonus of the tracheal chain was reduced by aspirin: 1.0 × 10⁻⁵ g/ml (○), 4.0 × 10⁻⁵ g/ml (●), 1.6 × 10⁻⁴ g/ml (△) and 0 (=control) ( ▲). In the aspirin-relaxed tracheal chain, the PGE₂-induced tonic contractions were plotted at the concentrations of lower than 4.0 × 10⁻⁸ g/ml, but the PGE₂-induced phasic contractions were plotted at concentrations higher than 1.6 × 10⁻⁷ g/ml. The magnitude of the phasic contractions was greater than that of the tonic contractions at higher concentrations of PGE₂ (See Fig. 2C). The broken line indicates the mean resting tonus in 81 experiments. Each point represents the mean of 3 experiments and the vertical bars indicate S.E.

FIG. 2. Effect of PGE₂ on the isolated guinea-pig tracheal chain with or without treatment of aspirin. PGE₂ 6.4 × 10⁻¹⁰ g/ml (A), 1.0 × 10⁻⁸ g/ml (B) or 6.4 × 10⁻⁷ g/ml (C) was added to an organ bath when the tracheal chain was at its normal resting tonus (left) or completely relaxed by aspirin 4.0 × 10⁻⁵ g/ml (right). Maximum relaxation was obtained by isoproterenol (IsP) 1.0 × 10⁻⁴ g/ml. W = washout.
FIG. 3. Effect of PGF$_2$α on the isolated guinea-pig tracheal chain after treatment with aspirin. PGF$_2$α was added to the organ bath after the resting tonus of the tracheal chain was reduced by aspirin: $1.0 \times 10^{-5}$ g/ml (●), $1.6 \times 10^{-4}$ g/ml (△) or 0 (=control) (○). The broken line indicates mean resting tonus in 66 experiments. Each point represents the mean of 3 experiments and the vertical bars indicate S.E.

PGF$_2$α also produced a persistent contraction in the tracheal chain relaxed by pretreatment with aspirin $1.0 \times 10^{-5}$ to $1.6 \times 10^{-4}$ g/ml (Figs. 3, 4). Elevation in the tonus was obtained by a low concentration of $1.0 \times 10^{-8}$ g/ml, and the resting tonus was restored by a concentration of $4.0 \times 10^{-8}$ g/ml which was ineffective on the normal resting tonus. In concentrations of $1.6 \times 10^{-7}$ to $2.5 \times 10^{-6}$ g/ml of PGF$_2$α, the tracheal contraction after pretreatment with aspirin was 4 to 6 times greater than that in normal state, and the magnitude of tracheal contraction produced by the same concentration increased as the concentration of aspirin was increased. The tracheal tonus reached 2.3 g, approximately 4 times the resting tonus, at a concentration of $2.5 \times 10^{-6}$ g/ml of PGF$_2$α after pretreatment with aspirin $1.6 \times 10^{-4}$ g/ml. However, the magnitude of contraction was progressively reduced with increases in concentrations of more than $2.5 \times 10^{-6}$ g/ml of PGF$_2$α, and the contraction produced by the highest concentration of $4.0 \times 10^{-5}$ g/ml was almost the same as that in the normal tracheal chain.

Effect of histamine on the tracheal tonus after pretreatment with aspirin: Histamine increased the tracheal tonus rapidly and dose-dependently, and the effect of histamine was stronger than that of PGF$_2$α, based on both dosage and intensity. Slight contraction occurred in a low concentration of $4.0 \times 10^{-8}$ g/ml, and the tracheal tonus was elevated to approximately 2.0 g at a concentration of $4.0 \times 10^{-5}$ g/ml (Fig. 5).

In the tracheal chain almost completely relaxed by aspirin $4.0 \times 10^{-5}$ g/ml, the response to histamine was also markedly enhanced and the tracheal tonus reached 3.0 g at a concentration of $4.0 \times 10^{-5}$ g/ml of histamine (Fig. 5). However, there was a little difference between histamine and PGF$_2$α. The potentiation of action occurred only at high concen-
trations of $2.5 \times 10^{-6}$ to $4.0 \times 10^{-5}$ g/ml of histamine. The contractions induced by $4.0 \times 10^{-8}$ to $6.4 \times 10^{-7}$ g/ml of histamine which were slightly effective in increasing the tonus in the normal state, did not reach or exceed the tonus in the normal tracheal chain.

**Effect of acetylcholine on the tracheal tonus after pretreatment with aspirin:** Acetylcholine also increased dose-dependently the tracheal tonus and was by far more active than histamine in regard to both dosage and intensity. Slight contraction occurred in a concentration as low as $1.0 \times 10^{-8}$ g/ml and the tracheal tonus exceeded 2.5 g at a concentration of $4.0 \times 10^{-5}$ g/ml of acetylcholine (Fig. 6).

On the other hand, pretreatment with $4.0 \times 10^{-3}$ g/ml of aspirin produced no potentiation of the action of acetylcholine. The dose-activity curves of acetylcholine were exactly the same with or without pretreatment of aspirin in concentrations of $1.6 \times 10^{-7}$ to $4.0 \times 10^{-5}$ g/ml (Fig. 6).

**Effect of PGF$_{2\alpha}$ on the tracheal chain after pretreatment with aspirin and PGE$_2$:** As stated above, the addition of PGE$_2$ $1.0 \times 10^{-8}$ to $4.0 \times 10^{-8}$ g/ml to the bath fluid restored the

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**Fig. 4.** Effect of PGF$_{2\alpha}$ on the isolated guinea-pig tracheal chain with or without treatment of aspirin. PGF$_{2\alpha}$ $4.0 \times 10^{-8}$ g/ml (A), $1.6 \times 10^{-7}$ g/ml (B), $2.5 \times 10^{-6}$ g/ml (C) or $4.0 \times 10^{-5}$ g/ml (D) was added to an organ bath when the tracheal tonus was at its normal resting tonus (left) or completely relaxed by aspirin $4.0 \times 10^{-5}$ g/ml (right). Maximum relaxation was obtained by isoproterenol (Isp) $1.0 \times 10^{-8}$ g/ml. W = washout.
FIG. 5. Effect of histamine on the isolated guinea-pig tracheal chain with or without treatment of aspirin. Histamine was added to an organ bath when the tracheal chain was at its normal resting tonus (○) or completely relaxed by aspirin $4.0 \times 10^{-5}$ g/ml (●). The broken line indicates the mean resting tonus in 45 experiments. Each point represents the mean of 3 experiments and the vertical bars represent S.E.

FIG. 6. Effect of acetylcholine on the isolated guinea-pig tracheal chain with or without treatment of aspirin. Acetylcholine was added to an organ bath when the tracheal chain was at its normal resting tonus (○) or completely relaxed by aspirin $4.0 \times 10^{-5}$ g/ml (●). The broken line indicates the mean resting tonus in 45 experiments. Each point represents the mean of 3 experiments and the vertical bars represent S.E.
tracheal tonus reduced by aspirin to the initial resting tonus. In the tracheal preparation, of which tonus was reduced by aspirin and then restored to the resting tonus by 1.0 × 10⁻⁸ g/ml of PGE₂, responses to PGF₂α in doses of more than 1.6 × 10⁻⁷ g/ml were still stronger than those in normal preparation, but the magnitude of contractions was clearly less than after pretreatment with aspirin alone (Fig. 7). The dose-activity curve of PGF₂α after treatment with aspirin 4.0 × 10⁻⁵ g/ml and PGE₂ 1.0 × 10⁻⁸ g/ml was intermediate between that of normal preparation and that of a preparation pretreated with aspirin alone. Moreover, the potentiation of action of PGF₂α was almost entirely abolished when the tracheal tonus reduced by aspirin was restored by a higher dose of PGE₂ 4.0 × 10⁻⁸ g/ml. That is, the dose-activity curve of PGF₂α after treatment with aspirin 4.0 × 10⁻⁵ g/ml and PGE₂ 4.0 × 10⁻⁸ g/ml coincided well with that in normal preparations.

**Effect of histamine on the tracheal chain after pretreatment with aspirin and PGE₂:**

The response to histamine in the tracheal preparation, of which tonus was reduced by aspirin 4.0 × 10⁻⁵ g/ml and then restored by PGE₂ 1.0 × 10⁻⁸ g/ml was approximately the same as in the tracheal chain pretreated with aspirin alone (Fig. 8). Thus, PGE₂ 1.0 × 10⁻⁸ g/ml had no effect on the potentiation of action of histamine by aspirin. However, a higher dose of PGE₂ 1.6 × 10⁻⁷ g/ml, which produced more than half-maximum reduction in the normal resting tonus, abolished the potentiation of the action of histamine, since the dose-activity curve of histamine after treatment with aspirin 4.0 × 10⁻⁵ g/ml and PGE₂ 1.6 × 10⁻⁷ g/ml
ml coincided with that in a normal state in concentrations of $2.5 \times 10^{-6}$ to $4.0 \times 10^{-5}$ g/ml.

Effect of aspirin on the tracheal chain after pretreatment with PGE$_2$, PGF$_2\alpha$, histamine and acetylcholine: Aspirin in concentrations of over $6.4 \times 10^{-3}$ g/ml produced a dose-dependent reduction in tonus of the isolated tracheal chain. The relaxation developed gradually and reached the maximum more than 30 minutes after addition of aspirin. The effect of aspirin was rather long-lived and recovery was incomplete even after washing out the drug.

After pretreatment with PGE$_2$ $1.0 \times 10^{-6}$ g/ml, which produced a marginal relaxation in the normal resting tonus, the addition of aspirin to the bath fluid had no relaxant effect on the tracheal preparation, and conversely, caused a slight increase in the tonus in concentrations which produced a dose-related relaxation of the resting tonus (Fig. 9).

Moreover, a clearly dose-dependent increase in the tracheal tonus was produced by aspirin after addition of PGF$_2\alpha$ $1.6 \times 10^{-7}$ g/ml to the bath fluid; an amount which had only marginal effect on the normal preparation. When aspirin $4.0 \times 10^{-5}$ g/ml was added to the bath fluid 10 minutes after pretreatment with PGF$_2\alpha$, the tracheal tonus of about 0.6 g increased to more than 1.6 g (Fig. 9).

On the other hand, pretreatment with histamine $1.0 \times 10^{-8}$ g/ml or acetylcholine $2.5 \times 10^{-9}$ g/ml, which produced a marginal contraction of the tracheal chain, did not affect the relaxant effect of aspirin.
DISCUSSION

The fact that PGE produces a relaxation of the isolated guinea-pig tracheal muscle was first demonstrated by Main (4) and has been confirmed by other workers (8). The results from the present investigation, however, show that PGE₂ produces not only a tracheal relaxation but also a tracheal contraction, and that the contractile effect of PGE₂ depends on the tonus level of the tracheal smooth muscles. In a normal state, PGE₂ was effective only in reducing the tonus in a dose-dependent way. When the tonus of the tracheal chain was reduced by treatment with aspirin, PGE₂ clearly elevated the reduced tonus of the preparation. The elevation in tonus was especially marked with low concentrations of PGE₂, and these concentrations had little effect on the normal resting tonus, and the PGE₂-induced contraction of the tracheal chain did not exceed the initial resting tonus. The magnitude and duration of contraction progressively diminished with increases in concentrations of PGE₂ which relaxed the resting tonus. The results suggest that only a small amount of PGE₂ plays an important role in the maintenance of the resting tonus, and any excess of PGE₂ may produce a reduction in the resting tonus. Therefore, the aspirin-induced reduction in the tracheal tonus was assumed to result from a reduction in the amount of intramural PGE₂. There was additional evidence to support this assumption. Pretreatment with PGE₂ prevented relaxation of the tracheal muscles induced by aspirin. That is, aspirin
produced no change in the resting tonus after pretreatment with a low concentration of PGE<sub>2</sub>, and this pretreatment was only marginally active in reducing the resting tonus but was sufficient to produce an elevation of the tracheal tonus reduced by aspirin. PGE<sub>2</sub> in the bath fluid may supply aspirin-depleted intramural PGE<sub>2</sub> to maintain the resting tonus.

On the other hand, PGF<sub>2α</sub> could not be the main participant in the maintenance of the resting tonus, because this prostaglandin produces only contraction regardless of the pre-existing level of the tracheal tonus. This assumption is also supported by the finding that bronchial tissue contains predominantly PGE<sub>2</sub> and only small amounts of PGFs (6). Histamine and acetylcholine may not be involved in the maintenance of the resting tonus, because pretreatment with these agonists in doses which were minimally effective on the resting tonus, did not affect the tracheal relaxant effect of aspirin. In support of this, we confirmed that neither atropine nor cyproheptadine reduced the resting tonus (data not shown).

Unlike PGE<sub>2</sub>, PGF<sub>2α</sub> produced a dose-related contraction of the normal tracheal chain, as was first demonstrated by Ånggård and Bergström (5). The present investigation indicates that the tracheal contractile effect of PGF<sub>2α</sub> is obviously potentiated by aspirin, and the peak contraction of PGF<sub>2α</sub> was much greater in magnitude in the tracheal preparation relaxed by aspirin than in the control tissue. The contractile effect of histamine but not of acetylcholine was also potentiated by aspirin pretreatment. It has been reported that stress on the tracheal muscle such as histamine-induced contraction or tracheal mucosa scratching releases more PGE<sub>2</sub> than PGF<sub>2α</sub> from the tracheal muscle (7, 9). As PGE<sub>2</sub> is a tracheal relaxant when the tracheal tonus is high, the release of PGE<sub>2</sub> from the tracheal muscle is probably a self-defensive mechanism protecting the muscle against overconstriction. In this investigation, the potentiation of the contractile effect of PGF<sub>2α</sub> and histamine was shown to be counteracted by doses of PGE<sub>2</sub> which reduced the resting tonus, though there was a subtle difference between PGF<sub>2α</sub> and histamine in that the potentiation of action of PGF<sub>2α</sub> by aspirin was easily reduced by the dose of PGE<sub>2</sub> which had little effect on the potentiation of action of histamine by aspirin. Therefore, the mechanism of the potentiation of tracheal contractile effect of PGF<sub>2α</sub> and histamine can be explained as follows: The tracheal contraction induced by either PGF<sub>2α</sub> or histamine releases intramural PGEs and the released PGEs counteract the PGF<sub>2α</sub> - or histamine-induced contraction. Aspirin interrupts this PGEs release, thus potentiating the PGF<sub>2α</sub> and histamine responses. This assumption could also account for the tracheal contractile effect of aspirin observed in the tracheal preparation pretreated with a low dose of PGF<sub>2α</sub>. This potentiation mechanism of PGF<sub>2α</sub> and histamine, mediators of bronchial asthma, may participate, at least in part, in the etiology of aspirin-induced asthma.

In conclusion, the present investigation suggests that PGI<sub>2</sub> plays an important role in the maintenance of the resting tonus of the isolated guinea-pig tracheal chain, and in large
doses, it also acts as a tracheal relaxant and attenuates the tracheal responses to PGF_{2\alpha} and histamine.

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