RELAXANT EFFECT OF ASPIRIN-LIKE DRUGS ON ISOLATED GUINEA PIG TRACHEAL CHAIN

Takaharu ONO, Minoru OHTSUKA, Shigeru SAKAI, Syuzo OHNO and Shigenobu KUMADA

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Yodogawabashi, Osaka 532, Japan

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Abstract—The interrelation of the inhibitory effect of aspirin-like drugs on the resting tonus of tracheal chain in guinea pigs, arachidonic acid-induced contraction in rat stomach fundus strips and bradykinin-induced bronchoconstriction in guinea pigs in vivo was investigated. All the drugs tested produced a dose-related inhibitory action on the resting tonus of the tracheal chain in comparatively low doses. Diclofenac was the most potent of all the drugs and was equal in activity to isoproterenol, followed in descending order by flufenamic acid, mefenamic acid, indomethacin, ibuprofen, phenylbutazone, oxyphenbutazone and aspirin. These aspirin-like drugs also inhibited arachidonic acid-induced contraction in rat stomach fundus strips. A highly significant correlation was observed between the potency of inhibition of the arachidonic acid-induced contraction and the relaxant effect on the tracheal chain. Moreover, the drugs antagonized bradykinin-induced bronchoconstriction in guinea pigs in vivo and the order of potency roughly paralleled that of the tracheal chain. These results suggest that the aspirin-like drugs produce a reduction in resting tonus of the isolated guinea pig tracheal chain by inhibition of intramural biosynthesis of prostaglandin endoperoxides.

Some non-steroidal anti-inflammatory drugs were found to be effective in inhibiting anaphylactic bronchoconstriction in guinea pigs in vivo (1). Based on these findings the authors attempted to determine the effect of aspirin-like drugs on the resting tonus in isolated guinea pig tracheal chain. Some noteworthy results were obtained; that is, the aspirin-like drugs reduced the resting tonus of the tracheal chain at relatively low doses, and diclofenac, which is the most potent of all the drugs used, was almost equal in activity to isoproterenol.

Aspirin-like drugs have been shown to inhibit prostaglandin (PG) biosynthesis in some 30 different systems (2, 3). The fact that many other pharmacologically active compounds such as morphine, major tranquilizers and antihistamines show no such inhibitory action suggests that inhibition of PG synthesis is a property peculiar to the aspirin-like drugs (4). Furthermore we and other authors have shown that PGs may be involved in maintaining the resting tonus of the tracheal chain (5, 6).

These findings prompted us to investigate the relationship between tracheal chain relaxation and inhibition of PG synthesis induced by aspirin-like drugs. The present study first demonstrates the potency of several aspirin-like drugs in reduction of the resting tonus of isolated guinea pig tracheal chain, and compares it with their inhibitory effect on contraction of isolated rat stomach fundus induced by arachidonic acid, which has been shown to act through conversion to PGs (7). Second, the inhibitory effect of the aspirin-like
drugs on bronchoconstriction induced by bradykinin, which has been considered to be a PG releaser (8), were investigated in guinea pigs in vivo.

MATERIALS AND METHODS

Guinea pig tracheal chain
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 being approximately 2 mm in width. Six tracheal chains were connected with fine thread to make a chain. The chains were suspended in a 25 ml organ bath containing Tyrode solution, which was aerated with a gas mixture of 95% oxygen and 5% carbon dioxide. The temperature of bath fluid was maintained at 37°C. The tracheal chain was connected to a force-displacement transducer under initial tension of 0.5-0.6 g, and its tonus was recorded isometrically on a polygraph. Isoproterenol 1.0×10^{-8} g/ml was added to the organ bath in advance to obtain the maximum relaxation, and the test compound was added to the bath. The concentration required to produce fifty percent reduction in tonus (ED50) was calculated by interpolation from the mean dose-response curve (effect by isoproterenol 1.0×10^{-8} g/ml = 100%).

Rat stomach fundus strip
The stomach was removed from Sprague-Dawley rats, weighing about 180 g, after the animals were fasted overnight. A strip of stomach fundus was suspended under initial tension of 0.6 g in a 10 ml organ bath containing Tyrode solution. The spasmogens employed were arachidonic acid 1.0×10^{-5} g/ml, PGE2 7.0×10^{-9} g/ml and PGF2α 8.0×10^{-8} g/ml. Aspirin-like drugs were added to the bath fluid 15 minutes before the spasmogens. The contraction induced by spasmogen was compared before and after addition of the aspirin-like drugs, and the concentration required to produce 50% inhibition was obtained by interpolation from the dose-activity curve. The other procedure was the same as described in the section under guinea pig tracheal chain.

Bradykinin- and PGF2α-induced bronchoconstriction in guinea pigs in vivo
Five male Hartley strain guinea pigs in each dose group were immobilized with 100 mg/kg i.p. of gallamine. The resistance of the lung to inflation imposed by artificial respiration was measured by the Konzett-Rössler method (9). The pressure of the air passing through the side arm of the tracheal cannula was recorded with a pressure transducer and polygraph. Bradykinin 16 μg/kg and PGF2α 250 μg/kg were injected i.v. The test drugs were also given i.v., and the bronchoconstriction induced by bradykinin and PGF2α after dosing with the aspirin-like drugs was compared with that during the predosing period.

Drugs
Drugs used were as follows: aspirin (Hoei), indomethacin (August Brandes), mefenamic acid (Parke Davis & Sankyo), flufenamic acid (Troponwerke), ibuprofen (Kaken), phenylbutazone (Fujisawa), oxyphenbutazone (Fujisawa), sodium diclofenac (Fujisawa), papaverine hydrochloride (Nakarai), gallamine triethiodide (Teikoku Kagaku), propranolol hydro-
chloride (Sigma), isoproterenol hydrochloride (Sigma), bradykinin triacetate (Sigma), arachidonic acid (Sigma), PGE\textsubscript{2} and PGF\textsubscript{2}r (Upjohn).

RESULTS

Effect of aspirin-like drugs on the resting tonus in isolated guinea pig tracheal chain

Potency: As shown in Fig. 1, all the aspirin-like drugs tested produced a dose-related reduction in tonus of the tracheal chain, and the dose-response curves of the drugs paralleled those of isoproterenol and papaverine. Diclofenac, flufenamic acid and mefenamic acid were of almost the same potency in reducing the tonus of the tracheal chain. The relaxant activity of these three drugs was obtained in concentrations ranging from $1.6 \times 10^{-10}$ to $1.0 \times 10^{-8}$ g/ml and was nearly equal to that of isoproterenol. Indomethacin and ibuprofen were ten to fifty times less effective than isoproterenol, but still more potent than papaverine. Phenylbutazone, oxyphenbutazone and aspirin were less potent than papaverine, but these three drugs produced complete relaxation at rather small concentrations of $2.5 \times 10^{-8}$ to $1.0 \times 10^{-5}$ g/ml. The tracheal relaxant effect of the aspirin-like drugs was not influenced by propranolol pretreatment.

Duration of effect: Aspirin-like drugs differed from isoproterenol and papaverine in onset of action. Isoproterenol and papaverine added to the bath fluid prompted an immediate relaxation of the resting tonus of the tracheal chain. Conversely, when the aspirin-like drugs were added to the fluid, the relaxation developed gradually to maximum over a period of more than 30 min (Fig. 2). Moreover, the relaxant effect of some of the aspirin-like drugs persisted after washing out. With regard to persistency, the aspirin-like drugs were divided into two groups. Diclofenac, indomethacin and aspirin were among the more persistent group. On the other hand, the relaxant effect of mefenamic acid was easily

Fig. 1. Dose-response curves for inhibition of resting tonus in isolated guinea pig tracheal chain by isoproterenol (••••••••), papaverine (○○○○○○), diclofenac (●●●●●●), flufenamic acid (■■■■■■), mefenamic acid (■■■■■■), indomethacin (∆∆∆∆∆∆), ibuprofen (▲▲▲▲▲▲), phenylbutazone (▲▲▲▲▲▲), oxyphenbutazone (■■■■■■) and aspirin (∆∆∆∆∆∆). Each point represents the mean of 6 experiments and vertical bars indicate the S.E. of the mean.
Fin. 2. Time course and reversibility of the relaxant effect on resting tonus in isolated guinea pig tracheal chains by (a) diclofenac: 1.6, 10 \textsuperscript{-10} g/ml, 6.4 \times 10 \textsuperscript{-10} \textsuperscript{-2.5} \times 10 \textsuperscript{-9} g/ml. (b) aspirin: 1.0 \times 10 \textsuperscript{-8} g/ml. (c) mefenamic acid: 2.5 \times 10 \textsuperscript{-9} g/ml, 1.0 \times 10 \textsuperscript{-8} g/ml. The drugs were left in contact with the preparations for 45 min and then washed out at the point indicated by the broken lines. Each point represents the mean of 6 determinations and vertical bars indicate the S.E. of the mean.

restored after washing out as was that of isoproterenol and papaverine. Flufenamic acid, ibuprofen, phenylbutazone and oxyphenbutazone were among the latter. The representative results are shown in Fig. 2.

Inhibitory effect of aspirin-like drugs on arachidonic acid-induced contraction in isolated rat stomach fundus strips

All of the aspirin-like drugs antagonized arachidonic acid-induced contraction of rat stomach fundus strips in a dose-related manner (Fig. 3). Diclofenac was the most potent of all the compounds tested, followed in descending order of potency by mefenamic acid, flufenamic acid, indomethacin, ibuprofen, phenylbutazone, aspirin and oxyphenbutazone. A highly significant linear correlation was found between -log ED50 values for inhibition of arachidonic acid-induced contraction of the rat fundus and those for relaxation of the tracheal chain (Fig. 4), although the ED50 values for each compound were not exactly the
FIG. 3. Dose-response curves of antagonism against arachidonic acid-induced contractions of isolated rat stomach strips by diclofenac (●—●), flufenamic acid (○—○), mefenamic acid (■—■), indomethacin (△—△), ibuprofen (▲—▲), phenylbutazone (▲—▲), oxyphenbutazone (■—■) and aspirin (△—△). Each point represents the mean of 6 experiments and vertical bars indicate the S.E. of the mean.

FIG. 4. Relationship between −log₁₀ LD₅₀ values of aspirin-like drugs for relaxation of isolated guinea pig tracheal chains and those for inhibition of arachidonic acid-induced contractions of isolated rat stomach strips. (●) diclofenac, (○) flufenamic acid, (■) mefenamic acid, (△) indomethacin, (▲) ibuprofen, (□) phenylbutazone, (●) aspirin, (○) oxyphenbutazone. Highly significant correlation (P < 0.01) was observed between the two parameters.

same in the two experiments. On the other hand, these compounds were entirely devoid of inhibitory effect on PGE₂ and PGF₂α-induced contractions at concentrations which completely inhibited the arachidonic acid-induced contraction (Fig. 5).

Antagonism to bradykinin-induced bronchoconstriction in guinea pigs in vivo

Potency: All of the aspirin-like drugs except oxyphenbutazone were effective in
FIG. 5. Effect of diclofenac (Dic) $6.4 \times 10^{-3}$ g/ml (A) and mefenamic acid (Mef) $2.5 \times 10^{-6}$ g/ml (B) on isolated rat stomach fundus contractions induced by arachidonic acid (AA) $1.0 \times 10^{-5}$ g/ml, PGE$_2$ $7.0 \times 10^{-8}$ g/ml and PGF$_2\alpha$ $8.0 \times 10^{-4}$ g/ml. The test drug was added 15 minutes before spasmogen.

FIG. 6. Antagonism against bradykinin-induced bronchoconstriction in guinea pig by flufenamic acid ( ), diclofenac ( ), mefenamic acid ( ), indomethacin (Δ ), phenylbutazone ( ), ibuprofen (△ ) and aspirin (△ ). Each point represents the mean of 5 determinations.

inhibiting bradykinin-induced bronchoconstriction, though all the drugs had no significant influence on the airway resistance to inflation. The inhibition produced by flufenamic acid, diclofenac, indomethacin and mefenamic acid was obtained with relatively small intravenous doses of 1-16 μg/kg, and dose-activity curves revealed a sharp upward inclination and easily exceeded fifty percent inhibition (Fig. 6). Thereafter the slope of the curve, however, leveled off and complete inhibition could barely be achieved even at dose of 4000 μg/kg. On the other hand, the inhibitory effect of the other drugs, phenylbutazone, aspirin and
Table 1. Potency ratios of the drugs to aspirin for anti-arachidonic acid and anti-bradykinin action

| Drugs          | Anti-arachidonic acid | Anti-bradykinin |
|----------------|-----------------------|-----------------|
| Diclofenac     | 0.005                 | 0.028           |
| Flufenamic acid| 0.016                 | 0.006           |
| Mefenamic acid | 0.014                 | 0.360           |
| Indomethacin   | 0.025                 | 0.034           |
| Ibuprofen      | 0.138                 | 0.800           |
| Phenylbutazone | 1.000                 | 0.460           |
| Oxyphenbutazone| 4.583                 | *               |
| Aspirin        | 1.000                 | 1.000           |

*: Oxyphenbutazone was ineffective in doses up to 4000 μg/kg.

Duration of action: The inhibition of bradykinin-induced bronchoconstriction occurred rapidly after intravenous administration of the aspirin-like drugs, with the peak effect occurring as soon as 2 minutes in most cases. Regarding duration of action, however, the aspirin-like drugs were divided into two groups. Flufenamic acid, mefenamic acid and phenylbutazone were of short duration, and the inhibition elicited by the drugs disappeared in as quickly as 15 minutes even at doses large enough to produce almost complete inhibition. On the other hand, the moderate to marked inhibition produced by intermediate and large doses of diclofenac, indomethacin, ibuprofen and aspirin was most persistent, although the slight inhibition by small doses of the compounds returned to normal shortly after dosing. Thus the results indicate that the drugs, which produced a persistent relaxation of the isolated tracheal chain, also displayed a persistent inhibition of bradykinin-induced bronchoconstriction.

DISCUSSION

All of the aspirin-like drugs employed in this investigation produced a dose-dependent relaxation of the resting tonus in isolated guinea pig tracheal chain. Diclofenac, flufenamic acid and mefenamic acid were almost as active as isoproterenol and were the most active...
of all the drugs tested, followed by indomethacin, ibuprofen and phenylbutazone. Aspirin and oxyphenbutazone were the least effective of all and were about 1,000 to 3,000 times less effective than diclofenac. As for indomethacin, a similar result has been reported (5). Since the effect of the drugs was not influenced by pretreatment with propranolol, the relaxation of the tracheal chain by the aspirin-like drugs does not result from stimulation of the beta-adrenergic receptors. Furthermore, the drugs also differed from isoproterenol and papaverine in being gradually effective. Thus it can be assumed that the relaxation induced by the aspirin-like drugs would not be due to any direct action on the smooth muscle, but rather to the interference of intramural synthesis of some chemical mediators which maintain the resting tonus of the tracheal chain.

The aspirin-like drugs also inhibited, in a dose-dependent manner, contraction of the isolated rat stomach fundus strips induced by arachidonic acid. Arachidonic acid is now known to be converted by cyclo-oxygenase of the PG synthetase system to the PG endoperoxides PGG$_2$ and PGH$_2$ (10, 11, 12), precursors of PGE$_2$ and PGF$_{2\alpha}$. The endoperoxides have contractile activity on the rat stomach fundus (13, 14). However, they have recently been shown to be transformed almost exclusively to prostaglandin X (PGX) in rat stomach fundus (15, 16), as in the arterial tissue of rabbits and pigs (16, 17). PGX also contracts the rat fundus, though somewhat less potently than the parent endoperoxides (15). Therefore, both PGX and the endoperoxides could be involved in the arachidonic acid-induced contraction in the rat fundus. Furthermore, a small amount of PGE$_2$ is also formed from arachidonic acid by rat stomach homogenates (18). As PGE$_2$ is more effective than PGG$_2$ and PGH$_2$ in causing contraction of the rat fundus (13, 14), the involvement of PGE$_2$ in the arachidonic acid-induced contraction would not be excluded. In this context, Splawinski et al have demonstrated in the experiment with SC-19220, a competitive antagonist of PGE$_2$ and PGF$_{2\alpha}$, that arachidonic acid, in doses up to concentration used in present investigation, PGE$_2$ and PGF$_{2\alpha}$ contract rat stomach fundus through stimulation of a common receptor (7). In present study, the aspirin-like drugs did not significantly antagonize the PGE$_2$ and PGF$_{2\alpha}$-induced contraction of the rat fundus, indicating that the inhibition by the aspirin-like drugs of the arachidonic acid-induced contraction is not caused at the receptor site. In addition, some of these drugs do not impair enzymic conversion from the endoperoxides to PGX (15). These findings indicate that the aspirin-like drugs antagonize the arachidonic acid-induced contraction of the rat fundus through inhibiting formation of the endoperoxides from arachidonic acid. Therefore, the highly significant correlation observed in this investigation between the potency of the aspirin-like drugs for the tracheal chain relaxation and that for the antagonism against the arachidonic acid-induced contraction in the rat fundus, suggests that the resting tonus of the guinea pig tracheal chain is reduced as the result of inhibition by the aspirin-like drugs of the generation of the endoperoxides.

This suggestion is further supported by the results that the aspirin-like drugs antagonized the bradykinin-induced bronchoconstriction in guinea pigs in vivo, and that the order of the antagonistic potency roughly paralleled the inhibitory effect on the arachidonic acid-induced
contraction of the rat fundus. Palmer et al have already reported that the bradykinin-induced bronchoconstriction is due to RCS (Rabbit aorta contracting substance) and PGF$_{2\alpha}$ released from the lung, and that aspirin, which inhibits their release, also antagonizes the bradykinin-induced bronchoconstriction (19). The activity of RCS is now thought to be due mainly to thromboxane A$_2$ (TXA$_2$), which is produced from the endoperoxides PGG$_2$ and PGH$_2$ in platelets and causes irreversible platelet aggregation (20). Recent work has shown that the endoperoxides are 7 to 9 times more potent than PGF$_{2\alpha}$ in causing contraction of the guinea pig trachea (13). In the light of these findings, the bronchoconstriction induced by bradykinin is considered to be due mainly to TXA$_2$ and the endoperoxides. In the present investigation, none of the aspirin-like drugs had any influence on the airway resistance to inflation, so the antagonism against bradykinin-induced bronchoconstriction cannot be attributed to broncholytic effect of the aspirin-like drugs. In addition, higher doses of aspirin do not significantly inhibit the effect of the endoperoxides (21) and RCS (8). Moncada et al. have recently shown that indomethacin and phenylbutazone are ineffective in affecting TXA$_2$-synthetase which converts the endoperoxides to TXA$_2$ (22). All these findings indicate that the antagonism against bradykinin could result from the inhibition by the aspirin-like drugs of the release (or synthesis) of the endoperoxides and TXA$_2$ from the lung by impairing the biochemical pathway generating the endoperoxides.

In the present investigation, there is one more intriguing finding. The aspirin-like drugs fell into two groups based on duration of action. Flufenamic acid, mefenamic acid and phenylbutazone were of short duration, their inhibitory effect on the bradykinin-induced bronchoconstriction almost disappearing in 30 minutes. Conversely, antagonism against bradykinin produced by diclofenac, indomethacin and aspirin steadily persisted over 60 minutes. Moreover a similar relationship between the two groups was observed in the isolated tracheal chain. The reduced resting tonus by flufenamic acid, mefenamic acid and phenylbutazone returned to the initial level shortly after washing out, whereas the washing-out displayed no immediate influence on the reduction in tonus produced by diclofenac, indomethacin and aspirin, though there was a slight trend toward recovery. The disparity of persistency of action between the two groups can hardly be explained from the biochemical point of view, since mefenamic acid and phenylbutazone which belong to the short-acting group as well as indomethacin and aspirin which belong to the long-acting group, have been similarly recognized as irreversible inhibitors of PG biosynthesis in vitro (3). Furthermore, this disparity cannot be attributed to differences in potency of the drugs, since complete inhibition by mefenamic acid and flufenamic acid was easily reversed after washing out, but the effects of indomethacin and diclofenac were hardly changed by washing out.

In conclusion, the present investigation suggests that the aspirin-like drugs reduce the resting tonus of the isolated guinea pig tracheal chain through inhibition of intramural biosynthesis of the PG endoperoxides.
REFERENCES

1) Takashima, T. and Hitomi, M.: *5th Int. Congr. Pharmacol.*, p. 1369, San Francisco, (1972)
2) Vane, J.R.: *Nature, New Biol.* **231**, 232 (1971)
3) Flower, R.J.: *Pharmacol. Rev.* **26**, 33 (1974)
4) Vane, J.R.: *Proc. 5th Int. Congr. Pharmacol.*, San Francisco 1972, Edited by Maxwell, R.A. and Acheson, G.H., Vol. 5, p. 352, Karger, Basel (1973)
5) Farmer, J.B., Farrar, D.G. and Wilson, J.: *Brit. J. Pharmacol.* **52**, 559 (1974)
6) Ono, T., Ohno, S. and Kumada, S.: *Folia pharmacol. japon.* **71**, 159P (1975) (in Japanese)
7) Splawinski, J.A., Nies, A.S., Sweetman, B. and Oates, J.A.: *J. Pharmacol. exp. Ther.* **187**, 501 (1973)
8) Piper, P.J. and Vane, J.R.: *Nature* **223**, 29 (1969)
9) Konzett, H. and Rösler, R.: *Arch. Pharmacol.* **195**, 71 (1940)
10) Hamberg, M. and Samuelsson, B.: *Proc. natn. Acad. Sci. U.S.A.* **70**, 899 (1973)
11) Nugteren, D.H. and Hazelhof, E.: *Biochim. Biophys. Acta* **326**, 448 (1973)
12) Hamberg, M. and Samuelsson, B.: *Proc. natn. Acad. Sci. U.S.A.* **71**, 3400 (1974)
13) Hamberg, M., Hedquist, P., Strandberg, K., Svensson, J. and Samuelsson, B.: *Life Sci.* **16**, 451 (1975)
14) Needleman, S., Moncada, S., Bunting, S., Vane, J.R., Hamberg, M. and Samuelsson, B.: *Nature* **261**, 558 (1976)
15) Gryglewski, R.J., Bunting, S., Moncada, S., Flower, R.J. and Vani, J.R.: *Prostaglandins* **12**, 655 (1976)
16) Moncada, S., Gryglewski, R.J., Bunting, S. and Vane, J.R.: *Prostaglandins* **12**, 715 (1976)
17) Moncada, S., Gryglewski, R., Bunting, S. and Vane, J.R.: *Nature* **263**, 663 (1976)
18) Pace-Asciak, C. and Wolfe, I.S.: *Biochim. Biophys. Acta* **218**, 539 (1970)
19) Palmer, M.A., Piper, P.J. and Vane, J.R.: *Brit. J. Pharmacol.* **49**, 226 (1973)
20) Hamberg, H., Svensson, T. and Samuelsson, B.: *Proc. natn. Acad. Sci. U.S.A.* **72**, 2994 (1975)
21) Hamberg, M., Svensson, J., Wakabayashi, T. and Samuelsson, B.: *Proc. natn. Acad. Sci. U.S.A.* **71**, 345 (1974)
22) Moncada, S., Needleman, P., Bunting, S. and Vane, J.R.: *Prostaglandins* **12**, 323 (1976)