STUDIES ON THE ACTION AND MECHANISM OF ACTION OF OXYFEDRINE ON ISOLATED TRACHEAL CHAINS¹

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Oxyfedrine, a β-sympathomimetic drug, did not affect isolated rat and rabbit trachea in concentrations from 2.86 \times 10^{-8} \text{ M} to 2.86 \times 10^{-4} \text{ M}, but on the guinea-pig trachea, it caused a dose dependent relaxation of natural tone in lower concentrations (1.79 \times 10^{-7} \text{ M} to 2.86 \times 10^{-6} \text{ M}). In higher concentrations (1.14 \times 10^{-5} \text{ M} to 2.86 \times 10^{-4} \text{ M}), however, a contraction was observed, which was also dose dependent. This contraction was not affected by atropine, lysargic acid diethylamide or by pretreatment with reserpine but was blocked by antihistamines (isothipendyl and clemastine). Adrenaline, noradrenaline, phenylephrine and isoprenaline did not contract the guinea-pig trachea, whereas contractions were observed after high concentrations of norephedrine, amphetamine, ephedrine and tyramine. These contractions were also unaffected by reserpine pretreatment.

It is concluded that the contraction of the guinea-pig trachea by oxyfedrine is related to its structural relationship to the phenylethylamines and might be due to histamine release, an action on histamine receptors or a histamine-like action.

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guinea pigs were used. Each trachea was cut into 6 rings. For each tracheal chain, 2 rings were taken from each trachea and were cut open before they were sewn into a chain.

2.1.2. Rats

Albino rats of either sex weighing on the average 300 g were used. 7 rats were used for preparing 3 tracheal chains. Each trachea was cut into 3 rings and for each tracheal chain 1 ring was taken from each trachea and was cut open before sewing together.

2.1.3. Rabbits

For the experiments, albino rabbits of either sex weighing between 2.5–3.5 kg were used. The trachea of each animal was cut into 6 rings which were then cut open and sewn together to a chain.

2.2. Solution

The tracheal chains were suspended in a 15 ml organ bath containing Krebs solution (Krebs, 1950). The solution was maintained at 37.5°C and was bubbled with a mixture of 95% O₂–5% CO₂.

2.3. Tissue responses

Tissue responses were recorded on a metal foil with an isotonic frontal writer. The load on the tissue was 1 g and the magnification was 8 fold. The tracheal chains were allowed to equilibrate for 3 hr before drugs were tested.

Most of the experiments were carried out with the cumulative method of Van Rossum and Van den Brink (1963). In this method, concentrations of oxyfedrine were increased 2 fold every 10 min without washing. On the addition of each concentration, the kymograph was switched off for 5 min and then switched on again for another 5 min.

The 3 tracheal chains prepared from 4 guinea pigs as described above, like the paired tracheal chains of Foster (1960), were identical in their reaction. One tracheal chain was therefore used as control for the other two.

Some experiments were carried out with the sequential method in which single doses of oxyfedrine were added to the bath for 10 min. At the end of this period, the tissue was washed at intervals of 20 min for a period of at least 2 hr before it was exposed to a second dose of oxyfedrine. (Oxyfedrine has a long duration of action. After a single high dose, the tissue requires up to 2 hr to recover from the effect of oxyfedrine.)

2.4. Pretreatment with reserpine

In experiments designed to test the indirect sympathomimetic effect of the drugs, guinea pigs were treated with reserpine, 5 mg/kg, injected intraperitoneally 24 hr before they were used. Some guinea pigs received reserpine, 2.5 mg/kg, at intervals of 48 hr and 24 hr before they were sacrificed for the experiments.

2.5. Drugs

The following drugs were used: oxyfedrine and isothipendyl hydrochloride (Chemiewerk Homburg); l-isopropylnarterenol and dichlorisopropylarterenol (Eli Lilly); d,l-isoprenaline (EGA Chemie); 1-nor-ephedrine (Knoll); reserpine (CIBA); l-phenylephrine HCl (Ch. Boehringer); noradrenaline and adrenaline (Hoechst); propranolol (Rhein-Pharma); tyramine hydrochloride (Dr. Th. Schuchardt); l-ephedrine hydrochloride, d,l-amphetamine and atropine sulfate (E. Merck); clemastine fumarate and lysergic acid diethyl-amide (Sandoz); serotonin creatinsulfate (C. Roth); acetylcholine iodide, histamine dihydrochloride (Fluka).

3. Results

3.1. Guinea-pig trachea

Experiments were performed on 60 tracheal chains. In the cumulative method, oxyfedrine, $1.79 \times 10^{-7}$ to $2.86 \times 10^{-6}$ M, caused a dose dependent relaxation of natural tone. When maximum relaxation was reached and the concentration of oxyfedrine was further increased, a contraction was observed. The concentration was also dose dependent and was observed from the cumulative dose of $1.14 \times 10^{-5}$ to $2.86 \times 10^{-4}$ M.

Single doses of oxyfedrine required 10 min to achieve maximum action. When the tracheal chains
were exposed to a single dose of $2.86 \times 10^{-4}$ M, in the sequential method, only very slight relaxation was observed which was followed by a contraction of the tracheal muscle.

In some experiments, the $2.86 \times 10^{-4}$ M dose caused no relaxations, but only contractions. Contraction were observed in all experiments performed with the cumulative as well as with the sequential method. After a single dose of $2.86 \times 10^{-4}$ M, the tracheal chains were washed at regular intervals for a period of at least 2 hr before they recovered from the effect of oxyfedrine. In most experiments dose-response curves to isoprenaline were first obtained, the tissue washed to recovery before responses to oxyfedrine were tested. In all experiments, isoprenaline caused only relaxation of the tracheal chains. The relaxation was observed from the dose $5.91 \times 10^{-10}$ M and reached a maximum at $1.89 \times 10^{-8}$ M. Further doses up to $4.73 \times 10^{-4}$ M did not contract the guinea-pig trachea (see fig. 1).

### 3.2. Rat trachea

18 tracheal chains were used for the experiments. Oxyfedrine tested in concentrations of $2.86 \times 10^{-8}$ to $2.86 \times 10^{-4}$ M had no effect on any of the tracheal chains. Also isoprenaline ($4.73 \times 10^{-9}$ to $4.73 \times 10^{-4}$ M) and histamine ($2.72 \times 10^{-5}$ M) did not affect the rat tracheal chains, whereas acetylcholine ($1.83 \times 10^{-5}$ M) caused a contraction.

### 3.3. Rabbit trachea

10 experiments were carried out with the rabbit trachea. Oxyfedrine ($2.86 \times 10^{-8}$ to $2.86 \times 10^{-4}$ M), isoprenaline ($4.73 \times 10^{-9}$ to $4.73 \times 10^{-4}$ M) and histamine ($2.72 \times 10^{-5}$ M) did not affect the tracheal chain whereas acetylcholine ($1.83 \times 10^{-5}$ M) caused a contraction.

### 3.4. Mechanism of action of the contraction of the guinea-pig trachea

The following experiments were performed to determine whether the contraction of the guinea-pig trachea by oxyfedrine was mediated through release of an endogenous stimulating substance (acetylcholine, 5-hydroxytryptamine, noradrenaline, histamine) or through an action on the receptors for these endogenous substances. At least 4 experiments were performed in each case.

### 3.5. Effect of atropine

Atropine ($1.44 \times 10^{-8}$ to $1.44 \times 10^{-4}$ M) did not affect contractions of the guinea pig trachea to oxyfedrine given cumulatively or sequentially; contractions to acetylcholine ($3.66 \times 10^{-8}$ M) were completely abolished by atropine ($1.44 \times 10^{-8}$ M).
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OX. 1.79 x 10^{-7} M

W

3.58 x 10^{-7}

1.43 x 10^{-6}

9.12 x 10^{-5}

4.56 x 10^{-5}

1.14 x 10^{-5}

OX. 1.79 x 10^{-7} M

ISOTH. 3.11 x 10^{-5} M

9.12 x 10^{-5}

7.16 x 10^{-7}

1.43 x 10^{-6}

W

4.56 x 10^{-5}

1.14 x 10^{-5}

Fig. 2. Effect of isothipendyl (ISOTH) on the contraction caused by oxyfedrine on the guinea-pig trachea. Upper tracing: control — relaxation and contraction by oxyfedrine (OX). Lower tracing: in the presence of isothipendyl (3.11 x 10^{-5} M). Oxyfedrine causes only relaxation. Each arrow denotes an increase of 2 fold in the concentration of oxyfedrine.

3.6. Effect of lysergic acid diethylamide

Lysergic acid diethylamide (3.09 x 10^{-7} to 3.09 x 10^{-6} M) did not affect contractions of the guinea-pig trachea to oxyfedrine given cumulatively or sequentially; contractions to 5-hydroxytryptamine (2.47 x 10^{-5} M) were blocked by lysergic acid diethylamide (3.09 x 10^{-7} M).

3.7. Effect of reserpine

Pretreatment of guinea pigs with reserpine did not affect contractions of the guinea-pig trachea to oxyfedrine.

3.8. Effect of antihistaminics

3.8.1. Isothipendyl

When the tracheal chains were exposed to isothipendyl (3.11 x 10^{-5} M) 30 min before cumulative doses of oxyfedrine were applied, relaxation only was observed. The contraction caused by oxyfedrine on the trachea was blocked (fig. 2).

3.8.2. Clemastine

Clemastine (3.41 x 10^{-5} M) added to the bath 30 min before oxyfedrine (2.86 x 10^{-4} M), abolished contractions of the guinea-pig trachea to oxyfedrine (fig. 3).
3.9. Contractions of the guinea-pig trachea by other sympathomimetics

Effects of other sympathomimetic amines on the guinea-pig trachea were determined.

3.10. Adrenaline, noradrenaline and phenylephrine

Adrenaline (5.46 X 10^{-8} to 5.46 X 10^{-6} M), noradrenaline (5.91 X 10^{-9} to 5.91 X 10^{-5} M) and phenylephrine (4.91 X 10^{-9} to 4.91 X 10^{-4} M) did not contract the guinea-pig trachea, even after blocking β-adrenoceptors with dichloroisoprenaline (3.57 X 10^{-6} to 3.57 X 10^{-5} M) and propranolol (3.86 X 10^{-6} to 3.86 X 10^{-5} M).

3.11. Norephedrine, ephedrine, amphetamine and tyramine

In contrast to adrenaline, noradrenaline and phenylephrine, contractions were observed after norephedrine, amphetamine, ephedrine and tyramine. In the sequential method, contractions were observed at the following concentrations: norephedrine 6.61 X 10^{-4} M, amphetamine 7.39 X 10^{-4} M, tyramine 5.76 X 10^{-4} M, and ephedrine 4.96 X 10^{-3} M.

As with oxyfedrine, single doses of these substances were tested at intervals of at least 2 hr. In the cumulative method, norephedrine, the substance used for the synthesis of oxyfedrine (Thiele et al., 1966), reacted similar to oxyfedrine. In lower concentrations (4.13 X 10^{-8} to 1.65 X 10^{-7} M) a relaxation was observed, while in higher concentrations (1.06 X 10^{-5} to 6.61 X 10^{-3} M) it caused a dose dependent contraction of the guinea-pig trachea. Amphetamine showed only very slight relaxing effect in cumulative doses (4.62 X 10^{-8} to 7.40 X 10^{-7} M); from the cumulative doses of 2.96 X 10^{-6} to 7.39 X 10^{-4} M, a dose dependent contraction was observed. Fig. 4 shows the effect of norephedrine and amphetamine on the guinea-pig trachea.

3.12. Effect of reserpine on the contraction

Since norephedrine, ephedrine, amphetamine and tyramine are indirectly acting sympathomimetics, effects of reserpine pretreatment on the contractions caused by these substances were determined. Pretreatment of guinea pigs with reserpine did not affect contractions of the guinea-pig trachea norephedrine, ephedrine, amphetamine and tyramine on the guinea-pig trachea.

4. Discussion

Oxyfedrine and isopropanaline did not affect tracheal preparations from the rat and rabbit. This agrees with the findings of Guigis (1969).

We also confirmed the report of Guigis (1969) and Akcasu (1959) that rat and rabbit trachea are insensitive to histamine, whereas the guinea-pig trachea is sensitive.

On the guinea-pig trachea, oxyfedrine caused a relaxation of the tracheal muscle in low concentrations, but a contraction in high concentrations. Isopropanaline, on the other hand, only relaxed the tracheal muscle. This confirms the findings of Habersang (1970).

Since contractions to oxyfedrine on the guinea-pig trachea were not affected by atropine, they were not
due to a release of acetylcholine or to an action on cholinoreceptors.

According to Vane (1960) and Kohli (1965), some sympathomimetics act on 5-hydroxytryptamine receptors. Such sympathomimetics also contract rabbit duodenum (Vane, 1960). However, in our experiments oxyfedrine did not contract the rabbit duodenum. Grobecker et al. (1972) reported that high concentrations of oxyfedrine release serotonin from thrombocytes, but since contractions of the guinea-pig trachea to oxyfedrine were not prevented by lysergic acid diethylamide they were unlikely to be due to an action on serotonin receptors or to release of serotonin from the guinea-pig trachea.

The presence of α-adrenoceptors in the guinea-pig trachea has been reported by some authors (Takagi et al., 1967; Chahl and O'Donnel, 1967, 1969; Everitt and Caincross, 1969; Guigris, 1969). In contrast, Foster (1963, 1966) was unable to demonstrate any α-adrenoceptors and classified the adrenoceptor in this organ as of the β-type.

In our experiments and under the same experimental conditions in which oxyfedrine contracted the guinea-pig trachea, no contractions were observed after adrenaline, noradrenaline, phenylephrine or isoprenaline. Even after blocking the β-adrenoceptors with dichlorisoprenaline or propranolol, these substances did not contract the guinea-pig trachea. Therefore the contraction to oxyfedrine could not be attributed to an effect on α-adrenoceptors.

In contrast to adrenaline, noradrenaline, phenylephrine and isoprenaline contractions were observed after high concentrations of amphetamine, norephedrine, ephedrine and tyramine without previous blockade of β-adrenoceptors. Foster (1963) also observed contractions of the guinea-pig trachea to amphetamine and ephedrine. Amphetamine, norephedrine, ephedrine and tyramine are known to be indirectly acting sympathomimetics.

Although Grobecker et al. (1972) reported that oxyfedrine and norephedrine caused a dose dependent reduction of noradrenaline in heart and brain of rats, pretreatment of guinea pigs with reserpine did not affect contractions caused by oxyfedrine and the above mentioned indirectly acting sympathomimetics, thus excluding an indirect sympathomimetic action.

Amphetamine, tyramine and phenylethylamine are also known to release histamine (Rothschild, 1962, 1966; Paton, 1957; Schmitt, 1964; Frimmer, 1969).

Norephedrine, the substance used for the synthesis of oxyfedrine (Thiele et al., 1966) is a metabolite of amphetamine (Smith and Dring, 1970) and it is possible that oxyfedrine and norephedrine also possess the ability to release histamine. This would appear to be borne out by the fact that the contractions caused by oxyfedrine were blocked by isothipendyl, a strong antihistaminic (Von Schlichtegroll, 1957) and by clemastine, an antihistaminic drug with a relatively high specificity (Frimmer, 1969).

Foster (1963) observed contractions of the guinea-pig trachea to dichloroisoprenaline and dichloronoradrenaline substances known to release histamine, whereas isoprenaline and noradrenaline do not (Rothschild, 1962, 1966). Rothschild attributed this difference to the strong lipophilic properties of the dichloro derivatives. Oxyfedrine also has a high lipid solubility (Beckett and Foster, 1972) and may be acting in the same way as dichloroisoprenaline and dichloronoradrenaline.

That oxyfedrine did not contract rabbit trachea, which are not sensitive to histamine, while it contracted the guinea-pig trachea, which is sensitive, is further indication that histamine may play a role in the contractions caused by oxyfedrine on the guinea-pig trachea.

From the foregoing, it is concluded, that the contraction of the guinea-pig trachea by oxyfedrine is related to its structural relationship to the phenylethylamines and might be due to histamine release, an action on histamine receptors or a histamine-like action. This action is observed only in very high concentrations which are not within the therapeutic range.

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