EFFECT OF ACETYLCHOLINE ON DESENSITIZATION OF ISOLATED RAT TRACHEA TO 5-HYDROXYTRYPTAMINE —A POSSIBLE ALLOSTERIC INTERACTION BETWEEN DRUG RECEPTORS

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It is well known that desensitization of smooth muscle preparations occurs by repeated exposure to a stimulating drug. This is called tachyphylaxis. This paper demonstrates the response of isolated rat tracheal muscle to 5-hydroxytryptamine (5-HT) as being diminished by repeated application in almost every instance and that 5-HT applied in the presence of stimulating drugs caused a further contraction when the preparation did not respond to 5-HT alone. For this reason interaction between 5-HT and other stimulating drugs was studied on the rat tracheal muscle after desensitization had occurred.

METHODS

After sacrificing a male rat (190 to 330 g in body wt.) by a blow on the neck, the trachea was removed and placed in a Petri-dish containing aerated Locke Ringer solution. After removing the adipose tissue, the trachea was incised longitudinally. The opened trachea was transversely cut along ring cartilages into 7 strips, which were then linked with thread (1). The tracheal muscle preparation was suspended in a 20 ml organ bath filled with Locke Ringer solution, kept at 32°C and bubbled with air. Responses of the preparation to drugs were isotonicity recorded through a writing lever (magnification $\times 6$, and weighting 300 mg). Locke Ringer solution used in this paper had the following composition (mM): NaCl, 154; KCl 5.6; CaCl_2 , 2.2; MgCl_2 , 2.1; NaHCO_3 , 5.9; glucose, 2.8.

All experimental results in this paper are expressed as mean of at least 8 experiments.

Drugs used: Agonists: acetylcholine chloride, Histamine hydrochloride, 5-hydroxytryptamine creatinine sulfate, 1-isoprenaline hydrochloride, KCl and phenylephrine hydrochloride. Antagonists: atropine sulfate, N-benzyl-5-methoxytryptamine hydrochloride (S-8), morphine hydrochloride, propranolol hydrochloride and tetrodotoxin. All drug concentrations refer to the salts and are given in g/ml.

FIG. 1. Desensitization of response of the isolated rat tracheal muscle to 5-HT.

FIG. 2. Antagonism between 5-HT and N-benzyl-5-methoxytryptamine (S-8).
TRACHEA, RAT (190 G ♂)

FIG. 3. Effects of isoprenaline (Iso), atropine (Atr) and tetrodotoxin (TTX) on the contraction produced by ACh or 5-HT.

RESULTS

1. 5-Hydroxytryptamine (5-HT)

The response to 5-HT was diminished by repeated application in almost every instance. A typical result is shown in Fig. 1, however, response to 5-HT was considerably inhibited by N-benzyl-5-methoxytryptamine hydrochloride (S-8) (2), an inhibitor of 5-HT (Fig. 2) and by isoprenaline (Fig. 2) but was little affected by tetrodotoxin (10^{-6} g/ml), atropine (3×10^{-7} g/ml) (Fig. 3) and morphine (10^{-3} g/ml). Relaxation of 5-HT-induced contraction by isoprenaline was competitively blocked by propranolol (Fig. 4).

In preparations where response to 5-HT was abolished by repeated application, 5-HT added in the presence of ACh resulted in a further contraction (Fig. 5). The action of

FIG. 4. Antagonism between isoprenaline (Iso) and propranolol (Prop).

FIG. 5. A further contraction produced by 5-HT in the presence of ACh or KCl. Experimental results were obtained after response to 5-HT had been abolished by repeated application.

5-HT in the presence of ACh was not desensitized by repeated application. Further contraction by 5-HT in the presence of ACh was inhibited by isoprenaline and N-benzyl-5-methoxytryptamine hydrochloride (S-8) (Fig. 6) but not by tetrodotoxin (Fig. 6) and morphine. The relaxing effect of isoprenaline was also competitively blocked by propranolol (Fig. 6), however, both the contraction by ACh and the further contraction by 5-HT were

FIG. 6. Effects of various drugs on a further contraction induced by 5-HT in the presence of ACh. Experimental results were obtained after response to 5-HT was abolished by repeated application.

upper: Antagonism between 5-HT and N-benzyl-5-methoxytryptamine HCl (S-8).

middle: Inhibition of 5-HT-induced contraction by isoprenaline (Iso) and antagonism between isoprenaline and propranolol (Prop).

lower: Effects of atropine (Atr), isoprenaline (Iso) and tetrodotoxin (TTX) on the 5-HT-induced contraction in the presence of ACh.

completely inhibited by atropine (Fig. 6), while the response to 5-HT in the absence of ACh was little affected by atropine.

When KCl (3×10^{-3} g/ml) replaced ACh, similar results were obtained, however a further contraction by 5-HT in the presence of KCl was not blocked by atropine (1×10^{-7} g/ml) (Fig. 5).

2. Acetylcholine (ACh)

Dose-response curve of ACh (1×10^{-7} to 3×10^{-5} g/ml) was displaced parallel toward its high concentrations by atropine (10^{-7} g/ml), indicating competitive antagonism, however, isoprenaline (up to 3×10^{-5} g/ml) was without any effect on the response to ACh.

3. Other drugs

Histamine (up to 3×10^{-5} g/ml) and phenylephrine (up to 3×10^{-4} g/ml) did not contract the rat tracheal muscle.

DISCUSSION

In the guinea pig ileum, contraction induced by 5-HT is mediated through two kinds of receptors (3). One, M-receptor, which is present in nervous structures, can be blocked by morphine, tetrodotoxin and atropine. The other, D-receptor, which is in the smooth muscle fibres, can be blocked by dibenzylamine and so-called 5-HT inhibitors. In this study response of the rat trachea to 5-HT was blocked by S-8, an inhibitor of 5-HT but not by tetrodotoxin, atropine and morphine. These facts suggest that response of the rat tracheal muscle to 5-HT was not mediated through the ACh-release but due to stimulation of 5-HT-receptor in the smooth muscle. It has been clearly demonstrated, however, by Offermeier and Ariens (4) that in guinea pig trachea response to 5-HT was mediated via the receptor in the cholinergic nerve structures. Difference between our results and the aforementioned in the site of action of 5-HT may be due to species difference.

In preparations where response to 5-HT was abolished by repeated application, further contraction by 5-HT in the presence of ACh was also mediated through 5-HT-receptor in the smooth muscle, as response to 5-HT in the presence of ACh was affected by antagonists in the same manner as that observed on preparations where tachyphylaxis did not occur. Since further contraction by 5-HT in the presence of ACh was abolished by atropine, it can be assumed that further contraction by 5-HT in the presence of ACh is concerned with activation of the ACh receptor, however, when KCl was used instead of ACh, a further contraction by 5-HT was also obtained. It can therefore be considered that muscle contraction produced by stimulating drugs induces conformational changes in the cell membrane and that new receptors for 5-HT are uncovered or that desensitized receptors of 5-HT have been resensitized.

The fact that isoprenaline inhibits only the contraction by 5-HT but not that by ACh suggests that the contraction process after formation of 5-HT-receptor complexes may be different from that after formation of ACh-receptor complexes.

SUMMARY

The response of the isolated rat trachea to 5-hydroxytryptamine (5-HT) was inhibited by N-benzyl-5-methoxytryptamine (S-8), an inhibitor of 5-HT and isoprenaline but not by tetrodotoxin, atropine and morphine, indicating that 5-HT acts via the 5-HT receptor in the smooth muscle. In the preparation where response to 5-HT was abolished by repeated exposure, 5-HT added in the presence of ACh or KCl caused a further contraction. A possible mechanism for this phenomenon was discussed in this paper.

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