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Serotonin
l-Noradrenaline-receptors
Serotonin antagonists
Serotonin receptors
Smooth muscles-serotonin interaction

Lack of Evidence for M- and D-Serotonin Receptors in the Cat's Isolated Tracheal Smooth Muscle

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Gaddum and *Picarelli* 1957, on the basis of experiments with some smooth musculature of isolated terminal ileum of guinea-pigs reached the conclusion that serotonin shows its effects by influencing two types of receptors in the smooth muscles: M-receptors and D-receptors, so-called because they are blocked by morphine and dibenziline (dihydroergotamine), respectively.

Apart from morphine, atropine, cocaine and methadone were indicated as blocking agents of serotonin M-receptors. Apart from dibenziline, dihydroergotamine, lysergic acid diethylamide (LSD_{25}), 2-bromlysergic acid diethylamide and 5-benziloxygramine were included as blocking agents of serotonin D-receptors. As the D-receptor blocking agents remove the serotonin effects not only on the guinea-pig ileum smooth muscle but also on the rat uterus smooth muscles and rabbit ear blood vessels, the conclusion was reached that smooth muscles contractions caused by serotonin are achieved through its interaction, mainly with D-receptors, found in the cells of respective smooth muscles.

Our aim was to investigate the effect of serotonin on the respiratory smooth muscles with a view to establishing whether the same mechanisms and receptors participate in obtaining serotonin effects in this type of smooth muscles.

Material and Methods

Experiments were made on 42 cats of both sexes, with an average weight of about 2800 g. Part of the trachea of the urethane anaesthetized cats was cut and prepared according to the method of *Castillo* and *De Beer* in *A. Akçasu's*

[1959] modification. We obtained the contraction of the tracheal smooth muscles, which are extremely sensitive to serotonin by freshly prepared serotonincreatinine sulphate solution applied to the water bath at a $1 \cdot 10^{-7}$ to $1 \cdot 10^{-6}$ concentration. As blocking agents of hypothetically existing serotonin M-receptors we used morphine hydrochloride and atropine sulphate (Pharmachim, Sofia), and as D-receptor blocking agents, dihydroergotamine (Dihydergot, Sandoz) and Lysergic acid diethylamide (Delysid, Sandoz). In addition, we used the beta-blocking agent Inderal (I.C.I., Gt Britain) and l-noradrenalin bitartrate (Farbenindustrie, Naumburg).

By the method of the smallest squares we found the equations of the regression straight lines which, inserted on the co-ordinate system, illustrate most accurately the relationship between the serotonin effects and some of the examined substances. In addition, by means of the Student-Fischer 't'-Test, we examined the statistical values of the 'a' and 'b' coefficients of the previously obtained equations of the regression straight lines with a view to establishing both the influence of dihydroergotamine and lysergic acid diethylamide on the serotonin effects and the possible differences in the degree of the latter. In the same way we analysed the influence of l-noradrenalin on the serotonin effects in order to be able to compare its inhibiting action with that of the examined substances. We found the dispersion around the regression straight lines by means of the 'F' test [*Bailey*, 1959].

Results

The atropine experiments showed that at a concentration of the order $1 \cdot 10^{-6}$ this alkaloid has no influence on the serotonin effects. Both the spasm and the rhythmical movement of the isolated smooth tracheal muscle, obtained as a result of the serotonin action, remained unchanged. Similar results were obtained in the morphine experiments, too. Introduced on the background even of comparatively large concentration ($1 \cdot 10^{-5}$) of morphine hydrochloride in the water bath, serotonin displays its customary spasmogenic action.

The analysis of the results of the influence of lysergic acid diethylamide, dihydroergotamine and l-noradrenalin on the serotonin effects (Table I and Fig. 1), shows the following:

1. We have no reason to consider that the lysergic acid diethylamide at a $1 \cdot 10^{-6}$ concentration has an influence on the contractions of an isolated cat tracheal smooth muscle caused by serotonin ('a' and 'b' coefficients are insignificantly different 'a' from 0 and 'b' from 1, respectively).

2. In serotonin contractions up to 10 mm, the dihydroergotamine effect at a $1 \cdot 10^{-6}$ concentration is zero, and in a contraction in the

order of about 20 mm, a minimum relaxation of about 1.6 mm is obtained (the 'a' coefficient is insignificantly different from 0, and the 'b' coefficient is considerably smaller than 1).

3. l-Noradrenalin has a strong inhibitory action on the serotonin effects (coefficient 'a' is considerably different from 0, and coefficient 'b' — from 1).

TABLE I

Results of the regression analysis of serotonin effects on cat's isolated tracheal muscles and the influences of Delysid (LSD_{25}), Dihydergot and l-noradrenalin

		Numbers of analyses	equations of regression straight lines	dispersion around the regression straight line	error of „b" coefficients	error of „a" coefficients
Substances x–y		N	y = a+b x	Syx	Sb	Sa
I. Serotonin ($1\cdot10^{-7}$–$1\cdot10^{-6}$)	Delysid ($1\cdot10^{-6}$)	8	y = 0.68+0.919x	1.04	0.06	0.77
II. Serotonin ($1\cdot10^{-7}$–$1\cdot10^{-6}$)	Dihydergot ($1\cdot10^{-6}$)	9	y = 0.64+0.886x	0.77	0.05	0.55
III. Serotonin ($1\cdot10^{-7}$–$1\cdot10^{-6}$)	Noradrenalin ($1\cdot10^{-6}$)	10	y = 3.49+0.637x	2.77	0.12	0.44

As can be seen in Fig. 1, the regression straight lines of lysergic acid diethylamide and dihydroergotamine are very close (coefficient 'b' is not considerably different). Therefore, it can be assumed that in the given concentrations these two substances have no expressed effect on the serotonin contractions. The 'b' coefficient of l-noradrenalin is considerably different from the coefficient 'b' of lysergic acid diethylamide and dihydroergotamine. In the same way the dispersion around the regression straight line is considerably bigger in the case of l-noradrenalin than in the cases of the two other substances.

The inhibitory effect of l-noradrenalin, remains the same on the background of the alpha-adrenolytic dihydroergotamine ($1\cdot10^{-6}$) but it is reduced considerably or is completely blocked after the preliminary introduction of the beta-adrenolytic Inderal® in the water bath at a $1\cdot10^{-6}-1\cdot10^{-5}$ concentration.

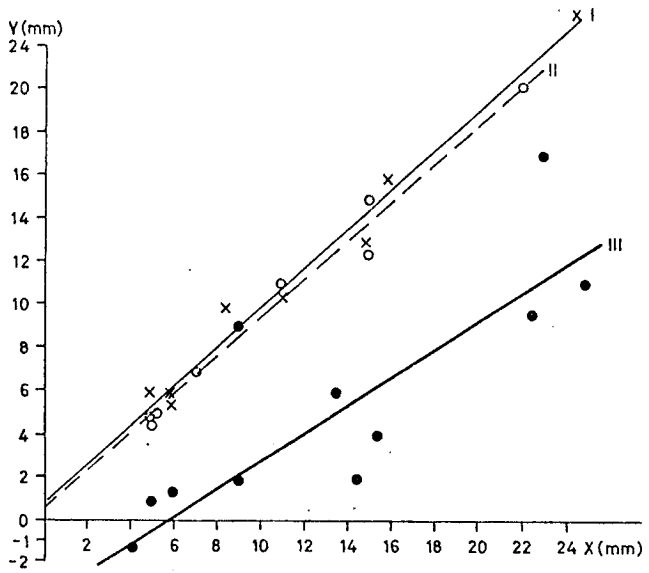

Fig. 1. Regression straight lines of the influence of Delysid (I ———), Dihydergot (II – – – –) and l-noradrenalin (III ———) on serotonin-induced contractions of cat's isolated smooth tracheal muscles. Relationships analysed according to the method of the smallest squares. On the abscissa (×), maximal serotonin contractions of trachea smooth muscles in mm; on the ordinate (Y)-serotonin contractions of trachea smooth muscles in mm, 1 min after Delysid $1 \cdot 10^{-6}$ (×), Dihydergot $1 \cdot 10^{-6}$ (○), or l-noradrenalin $1 \cdot 10^{-6}$ (·), are applied to the water bath.

Discussion

Our investigations showed that in the cat's isolated tracheal muscle, the serotonin effects were not accomplished by means of M- and D-receptors. In this respect they coincided with the results of *M. Wurzel*'s investigation [1966] who, in experiments with isolated rabbit aorta strip found that both morphine and lysergic acid diethylamide had no influence on the serotonin effects.

The acceptance of M- and D-receptor accomplishing serotonin effects in the smooth muscles is insufficiently justified. It is based on pharmacodynamic considerations, referring only to some types of smooth muscles. The lack of a molecular structural similarity between the agonist and the respective antagonists is also an argument against the concept that morphine and dibenzyline, dihydroergotamine and lysergic acid diethylamide, respectively, are

specific blocking agents of the serotonin receptors in the smooth muscles.

It is interesting that contrary to the classical views [1] in our investigations, l-noradrenalin proved to be a very strong activator of the beta-adrenergic receptors in the isolated cat tracheal muscles.

Summary

1. Serotonin effects upon isolated smooth muscles of cat trachea are not accomplished by means of the M- and D-receptors.

2. The inhibition of serotonin-induced contractions by l-Noradrenalin is due to activation of beta-adrenergic receptors.

3. l-Noradrenalin displays a very strong activating effect upon the beta-adrenergic receptors in the isolated smooth muscles of cat trachea.

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