Brit. J. Pharmacol. (1956), **11**, 289.

THE EFFECTS OF 5-HYDROXYTRYPTAMINE AND ITS ANTAGONISTS ON TIDAL AIR

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(RECEIVED APRIL 19, 1956)

Various reports suggest that 5-hydroxytryptamine (5HT) can cause bronchoconstriction. Thus, Reid and Rand (1952) described a bronchoconstrictor effect in the anaesthetized cat manifested by an increase in intrapleural pressure while respiratory excursions remained normal or decreased. Comroe, van Lingen, Stroud, and Roncoroni (1953) found that pressure-volume curves in anaesthetized cats suggested that large doses of 5HT caused bronchoconstriction. This was explained as mainly a direct constriction of the bronchial muscle, but it also appeared to be partly reflex in origin. Kottegoda and Mott (1955) noted that 5HT increased intratracheal pressure in the vagotomized cat and attributed this to direct bronchoconstriction. A direct effect of 5HT on bronchial muscle, independent of reflexes, has been observed in the perfused lungs of the rabbit (Freyburger, Gràham, Rapport, Seay, Govier, Swoap, and Van der Brook, 1952), cat (Gaddum, Hebb, Silver, and Swan, 1953) and guinea-pig (Bhattacharya, 1955). A constrictor effect of 5HT has also been noted in the tracheal chain of the guinea-pig (Freyburger *et al.*, 1952) and cat (Sinha and West, 1953). Furthermore, 5HT administered as an aerosol causes dyspnoea in the guinea-pig, resembling that produced by histamine or acetylcholine (Herxheimer, 1953a, 1953b, and 1955).

As yet, the effect of 5HT on tidal air has not been investigated in the intact and spinal animal. It therefore seemed of interest to make such an investigation with a view to obtaining further information on the mode of action. Another purpose of the study was to investigate whether agents found to be potent antagonists of 5HT in isolated organs *in vitro* also antagonize the bronchoconstriction induced by 5HT *in vivo*.

METHODS

Cats were anaesthetized with a mixture of chloralose (0.05 g./kg.) and urethane (0.5 g./kg.) administered subcutaneously. In the course of some experiments first the vagi were cut and later the spinal cord was sectioned at C 1. Guinea-pigs were anaesthetized by intraperitoneal administration of urethane (1.2 g./kg.). Spinal cats and guinea-pigs were also used, the operation being conducted under ether anaesthesia.

Changes in the distensibility of the lungs at constant pressure were recorded by means of the overflow method of Konzett and Rössler (1940). The lungs were ventilated by a Starling respiration pump driving air into the trachea. The maximum ventilation pressure was kept constant at 8–10 cm. of water. The excess air, which did not enter the trachea, raised a piston carrying a lever which recorded a vertical line on the kymograph. The lever rose during insufflation and fell when insufflation ended. A decrease in the volume of air entering the lungs caused the lever to rise further. Volume calibrations of the ventilation overflow were made in some but not in all experiments.

It may be objected that changes in tidal air, measured by this method, do not distinguish between bronchoconstriction and a displacement of air due to an increase of the volume of blood in the lungs (Barer and Nusser, 1953). It is extremely difficult to settle this question (Barer and Nusser, 1953). The fact that 5HT produced bronchoconstriction in perfused cat lungs without greatly changing the volume of blood in the venous reservoir (Gaddum, Hebb, Silver, and Swan, 1953) may be considered as evidence in favour of the decrease in tidal air being due to a bronchoconstrictor action of 5HT in the intact animal as well. Moreover, in order to produce a reduction in tidal air large volumes of saline (20–40 ml.) had to be injected intravenously (Barer and Nusser, 1953). The rapid injection of 10 ml. saline into the jugular vein had no influence on the tidal air in our experiments. It is improbable that 5HT could elicit such large blood volume changes in the lungs to produce, in this way, a reduction of tidal air. This point will be discussed later.

In some experiments on cats, especially those in which antagonists of 5HT were studied, the thorax was opened and the animal eviscerated. This procedure eliminated changes in intra-thoracic pressure due to variations in abdominal pressure. Moreover, it proved advantageous as 5HT can cause intestinal spasm and bowel evacuation (Erspamer, 1954). In addition, evisceration may be of importance in the

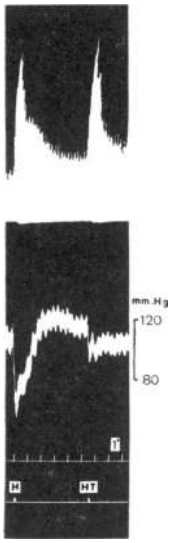

FIG. 1.—Reduction of tidal air induced by histamine (3.4 μg./kg. at H) and by 5-hydroxytryptamine (3.4 μg./kg. at HT) in the anaesthetized cat (2.9 kg.). Blood pressure recorded in the femoral artery. Time in min.

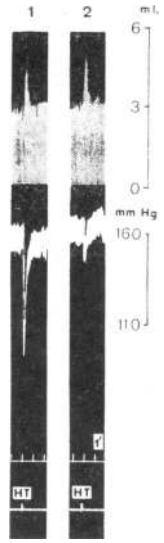

FIG. 3.—Reduction of tidal air induced by 5-hydroxytryptamine (16 μg./kg. at HT) in the anaesthetized cat (3 kg.) before (part 1) and after (part 2) section of both vagi. Blood pressure recorded in the carotid artery. Time in min.

study of 5HT-antagonists as, for instance, radioactive lysergic acid diethylamide is mainly excreted with the bile into the small intestine (Boyd, Rothlin, Bonner, Slater, and Hodge, 1955; Stoll, Rothlin, Rutschmann, and Schalch, 1955).

Systemic blood pressure was measured in the carotid or femoral artery of the cats; in a few experiments on cats, pulmonary arterial pressure was measured, by means of a water manometer, from a branch of one pulmonary artery.

The drugs studied were: 5HT-creatinine sulphate, histamine dihydrochloride, acetylcholine chloride (Roche), D-lysergic acid diethylamide tartrate (LSD), 1-acetyl-D-lysergic acid diethylamide bitartrate, 2-brom-D-lysergic acid diethylamide bitartrate, ergometrine tartrate, atropine sulphate, and 1-methyl-4-amino-*N'*-phenyl-*N'*-(2'-thenyl)-piperidine (Sandosten). All doses refer to the salts.

RESULTS

Effects in the Cat.—5HT produced a reduction of tidal air in 14 anaesthetized cats (Fig. 1) and in the 13 spinal cats. The threshold doses on both preparations ranged from 3–35 μg./kg. A quantitative comparison of the effect on tidal air in the anaesthetized cat and in the spinal cat, utilizing threshold doses, was therefore not profitable. The response to 5HT often increased very markedly during the first 30 min. of the experiment. Small

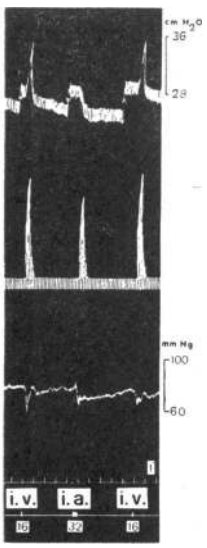

FIG. 2.—Reduction of tidal air induced by 5-hydroxytryptamine injected intravenously (16 μg./kg. at i.v.) and into the left auricle (32 μg./kg. at i.a.) of the anaesthetized cat (3 kg.). Above: pulmonary artery pressure recorded from a branch of the pulmonary artery by means of a piston recorder (lever adjusted at x). Below: blood pressure recorded in the femoral artery. Time in min.

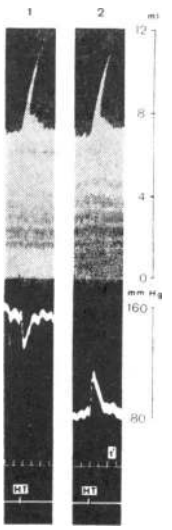

FIG. 4.—Reduction of tidal air induced by 5-hydroxytryptamine (32 μg./kg. at HT) in the anaesthetized and vagotomized cat (3 kg.) before (part 1) and after (part 2) section of the spinal cord at C 1. Blood pressure recorded in the carotid artery. Time in min.

FIG. 5.—Reduction of tidal air induced by 5-hydroxytryptamine (6 μg./kg. at HT) in the anaesthetized guinea-pig (0.65 kg.). The sensitivity to 5-hydroxytryptamine increases in the early part of the experiment.

doses of 5HT usually caused a short-lasting effect on tidal air similar to that of histamine (Fig. 1). 5HT was sometimes as effective as histamine but more often weaker. In good preparations, the effect of small doses of 5HT could be reproduced several times at intervals of 4–10 min.

In order to determine whether the reduction of tidal air produced by 5HT was related to the constrictor action of 5HT on the pulmonary vessels (Ginzel and Kottegoda, 1953; Gaddum *et al.*, 1953) pulmonary arterial pressure was recorded in 3 anaesthetized cats; 5HT was injected intravenously and into the left auricle alternately (Fig. 2). The intravenous injection of 16 μg./kg. 5HT caused a reduction of tidal air, an increase in pulmonary arterial pressure and a decrease in systemic blood pressure. Injection into the left auricle of 32 μg./kg. 5HT caused a reduction of tidal air comparable to that produced by 16 μg./kg. 5HT injected intravenously, a fall in pulmonary arterial pressure and a fall in systemic blood pressure. It may be concluded from this experiment that 5HT decreases tidal air irrespective of whether pulmonary arterial pressure is increased or not. The greater effect on the tidal air after intravenous injection in Fig. 2 may be due to the rise of pulmonary arterial pressure, or to an additional reflex mechanism elicited from receptors in the pulmonary arterial bed. However, in two other experiments in which pulmonary arterial pressure was recorded the reduction of tidal air was the same whether 5HT was injected into the left auricle or given intravenously.

To determine the part played by reflexes in the decrease of tidal air, the effect of 5HT was studied before and after vagotomy in 7 anaesthetized cats. The response to 5HT was not modified by vagotomy in 5 experiments and was slightly decreased in two. Fig. 3 shows an example; the effect of 16 μg./kg. 5HT was much the same before (part 1) and after vagotomy (part 2) in spite of the marked change of the blood pressure response.

The effect of section of the spinal cord at C 1 on the response to 5HT was also studied in 7 anaesthetized vagotomized cats. After cutting the spinal cord, the response to 5HT was not changed in 5 experiments, slightly decreased in one and slightly increased in one. Fig. 4 shows the effect of 32 μg./kg. 5HT on the vagotomized cat (part 1) and on the same animal after cutting the spinal cord at C 1 (part 2). The reduction of tidal air remains much the same in spite of the pronounced difference in blood pressure level and of the divergent effect of 5HT on the blood pressure.

Effects in the Guinea-pig.—5HT diminished tidal air in 9 anaesthetized and in 4 spinal guinea-pigs. The threshold doses on both preparations ranged from 3 to 22 μg./kg. As in cats, the response to 5HT often increased during the first 30 min. of the experiment (Fig. 5). 5HT was either as effective as histamine (Fig. 6) or weaker. Like histamine, 5HT usually produced a short lasting effect; sometimes, however, its action was prolonged (Fig. 7).

Antagonists of 5HT.—LSD, when given in doses one-third to twice as great as those of 5HT, exerted a specific antagonism towards the effect on tidal air induced by 5HT in 5 anaesthetized and 9 spinal cats and on 8 anaesthetized guinea-pigs (Fig. 6). The reduction in tidal air induced by histamine (Fig. 6), acetylcholine or pilocarpine was not affected by LSD in the smallest doses found to inhibit 5HT.

LSD not only prevented the reduction in tidal air by 5HT; it also abolished a prolonged 5HT-

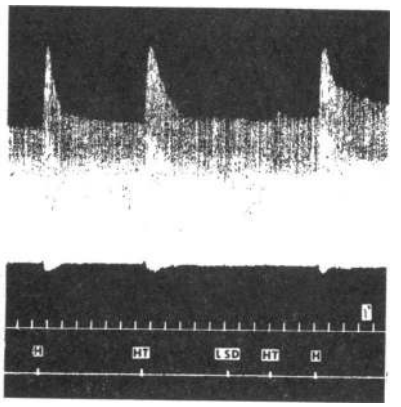

FIG. 6.—The specific antagonism of lysergic acid diethylamide (8.5 μg./kg. at LSD) towards the effect of 5-hydroxytryptamine (17 μg./kg. at HT) on the tidal air of the anaesthetized guinea-pig (0.6 kg.). The reduction in tidal air induced by histamine (17 μg./kg. at H) is not affected by lysergic acid diethylamide.

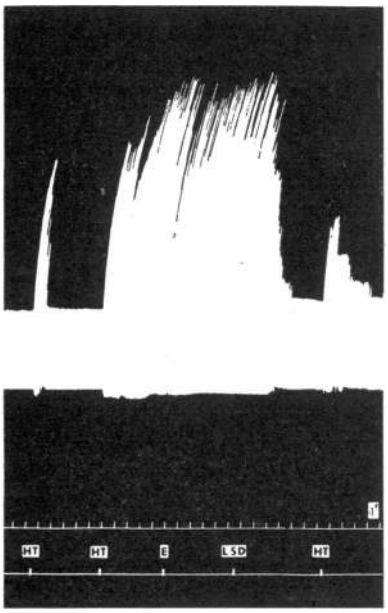

FIG. 7.—Reduction in tidal air induced by 5-hydroxytryptamine (10 μg./kg. at HT) and increase in tidal air induced by lysergic acid diethylamide (20 μg./kg. at LSD) in the anaesthetized guinea-pig (0.5 kg.). Ergometrine (200 μg./kg. at E) has practically no effect.

effect. An example is shown in Fig. 7; 5HT, 10 μg./kg. caused first a short-lasting and then a long-lasting reduction of tidal air in a guinea-pig. LSD, 20 μg./kg., completely abolished this effect, while 200 μg./kg. ergometrine had practically no effect. A subsequent injection of 5HT again caused a decrease in tidal air which was, however, somewhat less than before LSD.

1-Acetyl-LSD and 2-brom-LSD were as active as LSD in antagonizing the effects of 5HT on the tidal air in 4 anaesthetized and 5 spinal cats. In

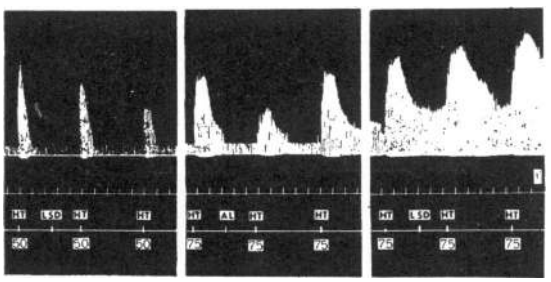

FIG. 8.—Antagonism of lysergic acid diethylamide (32 μg./kg. at LSD) and of 1-acetyl-lysergic acid diethylamide (32 μg./kg. at AL) to the reduction of tidal air by 5-hydroxytryptamine (given at HT; the amount given is indicated in μg./kg.) in the eviscerated spinal cat (3 kg.). The interval between the first part of the experiment and the second is 50 min., that between the second and third part is 12 min.

Fig. 8 the effects of LSD and acetyl-LSD on the spinal cat are compared. In the first part of the experiment 32 μg./kg. LSD diminished the effect of 5HT, the action of LSD being slow to develop but lasting a long time. The amount of 5HT had to be increased from 50 μg./kg. to 75 μg./kg. in order to obtain a response similar to that obtained before LSD. In the second part of the experiment 32 μg./kg. acetyl-LSD produced a short-lasting and partial inhibition of the effect of 5HT. In the third part of the experiment 32 μg./kg. LSD had no effect on the 5HT-induced reduction of tidal air. During this experiment, as in others, some tachyphylaxis developed. A comparison of LSD and 2-brom-LSD in the spinal cat is depicted in Fig. 9. The effect on the tidal air induced by 18 μg./kg. 5HT was partly diminished within 2 min. of giving 18 μg./kg. 2-brom-LSD and even

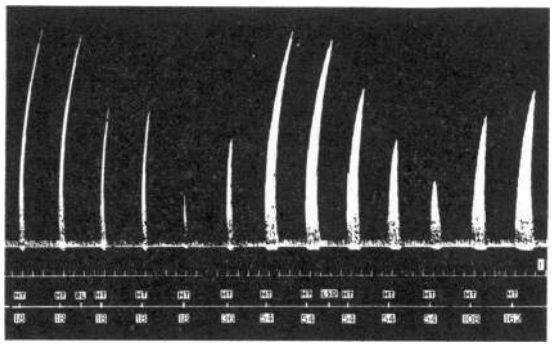

FIG. 9.—Antagonism of 2-brom-lysergic acid diethylamide (18 μg./kg. at BL) and of lysergic acid diethylamide (18 μg./kg. at LSD) to the reduction of tidal air induced by 5-hydroxytryptamine (given at HT; the amount given is indicated in μg./kg.) in the eviscerated spinal cat (2.7 kg.). The amount of 5-hydroxytryptamine given has to be increased during the experiment.

more 10 min. later. By increasing the amount of 5HT it was possible to obtain a response approximately identical with that obtained before 2-brom-LSD. The effect of 54 μg./kg. 5HT—three times the initial amount—was diminished by 18 μg./kg. LSD.

Atropine, in amounts greater than 300 μg./kg. diminished the effect of 5HT on the tidal air in 3 spinal and 1 anaesthetized cats. However, this antagonism was not specific as the effect of acetylcholine on tidal air was inhibited by atropine in far smaller amounts (15–40 μg./kg.) which did not interfere or even enhance the effect of 5HT.

The antihistamine 1-methyl-4-amino-*N'*-phenyl-*N'*-(2'-thenyl)-piperidine ("Sandosten") specifically inhibited the effect of histamine on the tidal air when given in small amounts (80–150 μg./kg.) but did not modify the response to 5HT. When

given in greater amounts (300 μg./kg.) it also diminished the effect of 5HT on the tidal air of 3 spinal cats.

Discussion

5HT decreased the tidal air of cats and guinea-pigs when measured under positive pressure respiration at constant stroke volume. The threshold doses of 5HT to produce this effect varied greatly in both spinal and anaesthetized animals. In general, the reduction of tidal air due to 5HT was short-lasting, resembling the action of histamine. 5HT, however, was usually less potent in cats and guinea-pigs than histamine in reducing the tidal air.

A reduction in tidal air occurs in the anaesthetized cat, whether 5HT is injected intravenously or into the left auricle. In the latter case there is no increase in pulmonary arterial blood pressure. The reduction in tidal air is therefore produced by 5HT independently of its action on the pulmonary arterial pressure.

If the reduction in tidal air were due only or mainly to pulmonary congestion, it would be surprising that the immediate large blood volume changes necessary for an identical reduction in tidal air should occur at such varied blood-pressure levels as are observed in the anaesthetized, vagotomized and spinal cat. Another point in favour of direct action on the bronchial muscles is that histamine had a similar action on the tidal air to 5HT in all experiments in which both substances were given.

Lysergic acid diethylamide (LSD) has been found a potent and specific antagonist of the effect of 5HT in reducing the tidal air of cats and guinea-pigs. This is in agreement with observations on the perfused lungs of cats (Gaddum, Hebb, Silver, and Swan, 1953) and of guinea-pigs (Bhattacharya, 1955), as well as in unanaesthetized guinea-pigs (Herxheimer, 1953b ; 1955). 1-Acetyl-LSD and 2-brom-LSD were about as potent as LSD in cats ; they are also at least as active as LSD in inhibiting the effect of 5HT on the isolated rat uterus and on the isolated rat kidney (Cerletti and Rothlin, 1955 ; Cerletti and Konzett, 1956). Our results demonstrate that, under suitable conditions, those compounds which exhibit an anti-5HT action on plain muscle *in vitro* may act similarly *in vivo*. However, it must be mentioned that LSD, 1-acetyl-LSD and 2-brom-LSD sometimes lose their antagonism to 5HT quite rapidly during an experiment. The reason for this tachyphylactic reaction is not clear. Ergometrine, even in high doses, does not antagonize the effect of 5HT on tidal air.

Atropine also exhibited a remarkable antagonism to the 5HT-effect on tidal air in cats. This is in agreement with the observations of Herxheimer (1953a, 1953b, and 1955), in unanaesthetized guinea-pigs, that atropine effectively antagonized the shock syndrome elicited by an aerosol of 5HT. However, atropine exerted a more specific antagonism towards acetylcholine ; this could be elicited by smaller doses than those required to antagonize the effects of 5HT.

High doses of the antihistamine 1-methyl-4-amino-*N'*-phenyl-*N'*-(2'-thenyl)-piperidine ("Sandosten") also antagonized to some degree the reduction of tidal air induced by 5HT. However, the antihistaminic action of this compound was more specific than its antagonism of 5HT. It may be noted that this antihistamine exerts a pronounced peripheral atropine-like action (Rothlin and Cerletti, 1955). This may be of significance in its antagonism to 5HT, as Herxheimer (1955) found the antihistamine promethazine, which also has marked atropine-like properties, to be an effective antagonist of 5HT, whereas the antihistamine mepyramine, which exerts little atropine-like action, does not protect against 5HT.

Since the reduction of tidal air after 5HT is most probably and mainly due to a direct bronchoconstrictor effect, the antagonistic action of the compounds studied should occur directly on the bronchial muscles and not by interruption of any reflex arc.

Summary

1. 5-Hydroxytryptamine (5HT) produces a decrease of tidal air at constant stroke volume in anaesthetized cats and guinea-pigs, as well as in spinal cats and guinea-pigs. The effect in anaesthetized cats is independent of the action of 5HT on the pulmonary arterial pressure.

2. In anaesthetized cats, the reduction of tidal air due to 5HT is usually identical before and after vagotomy, as well as before and after section of the spinal cord at C 1, suggesting mainly a direct, and not a reflex, action.

3. There are good reasons to suppose that 5HT reduces tidal air by a bronchoconstrictor action.

4. D-Lysergic acid diethylamide, 1-acetyl-D-lysergic acid diethylamide and 2-brom-D-lysergic acid diethylamide specifically antagonize the effect of 5HT on tidal air.

5. Atropine and the antihistamine 1-methyl-4-amino-*N'*-phenyl-*N'*-(2'-thenyl)-piperidine ("Sandosten") also partly antagonize the action of

5HT on tidal air, but less specifically than they diminish the bronchoconstrictor action of acetylcholine and histamine, respectively.

References

Barer, G. R., and Nusser, E. (1953). *Brit. J. Pharmacol.*, **8**, 315.

Bhattacharya, B. K. (1955). *Arch. int. Pharmacodyn.*, **103**, 357.

Boyd, E. S., Rothlin, E., Bonner, J. F., Slater, I. H., and Hodge, H. C. (1955). *J. Pharmacol.*, **113**, 6.

Cerletti, A., and Konzett, H. (1956). *Arch. exp. Path. Pharmak.*, **228**, 146.

—— and Rothlin, E. (1955). *Nature, Lond.*, **176**, 785.

Comroe, J. H., jr., van Lingen, B., Stroud, R. C., and Roncoroni, A. (1953). *Amer. J. Physiol.*, **173**, 379.

Erspamer, V. (1954). *Pharmacol. Rev.*, **6**, 425.

Freyburger, W. A., Graham, B. E., Rapport, M. M., Seay, P. H., Govier, W. H., Swoap, O. F., and Van der Brook, M. J. (1952). *J. Pharmacol.*, **105**, 80.

Gaddum, J. H., Hebb, C. O., Silver, A., and Swan, A. A. B. (1953). *Quart. J. exp. Physiol.*, **38**, 255.

Ginzel, K. H., and Kottegoda, S. R. (1953). Ibid., **38**, 225.

Herxheimer, H. (1953a). *J. Physiol.*, **120**, 65P.

—— (1953b). Ibid., **122**, 49P.

—— (1955). Ibid., **128**, 435.

Konzett, H., and Rössler, R. (1940). *Arch. exp. Path. Pharmak.*, **195**, 71.

Kottegoda, S. R., and Mott, J. C. (1955). *Brit. J. Pharmacol.*, **10**, 66.

Reid, G., and Rand, M. (1952). *Nature, Lond.*, **169**, 801.

Rothlin, E., and Cerletti, A. (1955). *Ann. Allergy*, **13**, 80.

Sinha, Y. K., and West, G. B. (1953). *J. Pharm.*, **5**, 370.

Stoll, A., Rothlin, E., Rutschmann, J., and Schalch, W. R. (1955). *Experientia*, **11**, 396.