Brit. J. Pharmacol. (1957), 12, 410.

THE ACTION OF 5-HYDROXYTRYPTAMINE AND SOME OF ITS ANTAGONISTS ON THE UMBILICAL VESSELS OF THE HUMAN PLACENTA

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(RECEIVED APRIL 9, 1957)

The vasoconstrictor action of 5-hydroxytryptamine (5-HT) in the human placental preparation is about 10 times stronger than that of adrenaline and is antagonized by anti-adrenaline compounds like phentolamine. Both 5-HT and adrenaline are antagonized by yohimbine and chlorpromazine. Specific and strong anti-5-HT action is demonstrated for lysergic acid diethylamide (LSD) and tryptamine. Both LSD and tryptamine in larger doses have a vasoconstrictor action. Mescaline has no certain modifying effect on the action of 5-HT, but itself causes vasoconstriction in large doses. The antihistamine drug phenbenzamine in histamine blocking doses abolishes the action of 5-HT in half the preparations tested. The ganglionic blocking agent trimetaphan in large doses antagonizes the action of 5-HT added subsequently, and also, to a lesser degree, the effect of adrenaline. Hexamethonium and tetraethylammonium bromides are ineffective in this preparation. No certain modifying action of reserpine on subsequently added 5-HT could be demonstrated, and the same was true for heparin even in very high concentrations.

5-Hydroxytryptamine (5-HT) continues to attract considerable attention as a possible factor in different experimentally produced psychotic conditions in animals and as a compound possessing vascular effects. Although 5-HT has rather weak and inconsistent effects upon the systemic blood pressure in different animals and in man (Erspamer, 1954), evidence is accumulating for its strong vasoconstrictor action in certain local vascular beds.

In a previous report from this laboratory (Eliasson and Åström, 1955), the pharmacological actions of a series of compounds were studied on the blood vessels of isolated human placentas. 5-HT was included in this series, and its actions were compared with those of adrenaline and noradrenaline. It was shown that 5-HT, in the same manner as the catechol derivatives, caused a constriction of the umbilical vessels and its potency in this respect was about 10 times that of adrenaline. 5-HT showed many pharmacological similarities to adrenaline and noradrenaline; thus its action in this preparation was abolished by dihydroergotamine and enhanced by cocaine. The same ratio between the potency of 5-HT and adrenaline has been demonstrated in the lung vessels of the dog and the cat by Ginzel and Kottegoda (1953).

In the experiments here the action of 5-HT on the placenta has been studied further and special consideration has been given to a series of drugs known to antagonize the actions of 5-HT in other preparations. Since the umbilical vessels do not receive any nerve supply (Schmitt, 1922; Guarna, 1934) it was felt that a study of 5-HT antagonists in this preparation might yield information of interest concerning the pharmacological mechanisms involved.

METHODS

Human placentae were obtained from the hospital as soon as possible after delivery. The vessels of the umbilical cord were dissected free and cannulae were inserted and tied in the vein and in one of the anastomosing arteries about 2 to 3 cm. from the insertion of the cord at the surface of the placenta. The organ was placed in Ringer solution at 37°. Perfusion by means of a Dale-Schuster pump was performed with Tyrode solution to which, in half the preparations, 15% (v/v) of a solution of 6% dextran and 0.9% NaCl (Pharmacia) had been added. The presence of dextran in the perfusion liquid was effective in preventing oedema formation but did not influence the pharmacological effects of the drugs tested. The perfusion fluid was aerated with 6.5% carbon dioxide in oxygen. The *p*H of the perfusion liquid measured on the outflow side was 7.3 to 7.4.

The pump frequency was set at 46 strokes/min. and the stroke volume at the beginning of an experiment

was adjusted so as to yield a perfusion pressure of 60 to 80 mm. Hg. The stroke volume invariably had to be increased during the following 15 to 30 min. as more and more of the vessels opened up. After 20 to 30 min., the sensitivity of the preparation towards adrenaline was tested, and, if good and reproducible results were obtained, the tests were commenced. From the beginning of the tests, the stroke volume of the pump was kept constant. The venous outflow was not reperfused and was led off by a rubber tube, the outlet end of which was level with the placenta. The flow observed ranged from 20 to 40 ml./min. The inflow pressure was measured and recorded by a mercury manometer. Since the output from the pump was kept constant during the determination period, increases in resistance (vasoconstriction) in the placenta could be recorded as pressure changes.

The placenta preparation showed considerable variation in sensitivity. Not only was this true for different preparations but also for the same preparation during the course of one experiment (Eliasson and Åström, 1955). A total of 81 preparations were perfused, and of these 33 had to be discarded for quantitative comparisons since they were either too insensitive or gave varying responses to repeated tests with constant doses of adrenaline.

Of the substances used the following were kindly supplied by pharmaceutical manufacturers : chlorpromazine and hexamethonium bromide, May and Baker; dextran, Pharmacia; heparin, Vitrum; 5-hydroxytryptamine creatinine sulphate (5-HT), Abbott; lysergic acid diethylamide tartrate (LSD), Sandoz; phenbenzamine (Lergitin), Recip; phentolamine (Regitin) and reserpine, Ciba; tetraethylammonium bromide, Astra; trimetaphan (Arfonad), Hoffman-La Roche.

Results

The Action of 5-HT.—The action of 5-HT on the umbilical vessels is usually purely constrictor. However, in about 1 out of 5 preparations a diphasic response can be observed. In such preparations the pressure, after an initial rise, shows a secondary fall below control level (Fig. 4A). This effect is commonly present only at the beginning of an experiment and is also sometimes observed, although less frequently, with adrenaline and noradrenaline under similar conditions.

The vasoconstrictor action of 5-HT is very strong and doses of 5 to 10 μg. often cause complete closure of the vessels. In doses that give a moderate pressor response (0.5 to 3 μg.), 5-HT does not alter the effect of adrenaline added subsequently.

Repeated doses of 5-HT in the placenta preparation usually produce the same pressor effect if the intervals between injections are long enough to permit a complete return of the pressure to the original level. However, in 2 out of 8 preparations in which this phenomenon was particularly studied, the effect of subsequent doses was slightly less than that of the first dose. In both these preparations a longer time was required for recovery than in the other 6 experiments; this was true also after test doses of adrenaline.

Tryptamine.—In doses of 2.5 to 10 μg., which were 2 to 5 times greater than the dose of 5-HT which produced a good pressor response, tryptamine caused no effect on the perfusion pressure. These doses sufficed, however, to abolish completely the action of 5-HT added subsequently (Fig. 1). The effect of adrenaline after the tryptamine administration was usually unchanged,

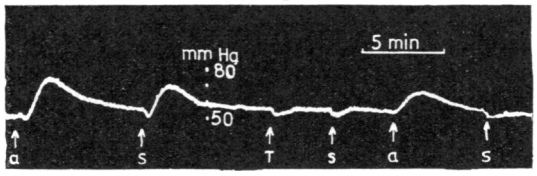

FIG. 1.—Record of umbilical arterial pressure in the isolated perfused human placenta showing the antagonistic action of tryptamine on 5-HT. The effect of adrenaline was slightly decreased after tryptamine in this preparation. a=adrenaline, 10 μg.; s=5-HT, 1 μg.; T=tryptamine, 5 μg.

although a slight decrease, as in Fig. 1, was observed in 2 out of 6 preparations. The histamine effect was unaffected by tryptamine. The preparation remained insensitive to 5-HT for a fairly short time, and normal responses could again be obtained 15 to 30 min. after the tryptamine administration.

Larger doses of tryptamine caused vasoconstriction. The activity in this respect was 10 to 20 times less than that of 5-HT.

Lysergic Acid Diethylamide.—LSD in doses of 5 to 25 μg. (1 to 5 times larger than the dose of 5-HT required to produce a good pressor response) had a constrictor action on the umbilical vessels in 2 out of 5 preparations. Whether LSD alone had any effect upon the perfusion pressure or not, the doses used always completely abolished the action of 5-HT. In 2 preparations the action of 5-HT was actually reversed after LSD. Administration of adrenaline and histamine before and after LSD gave identical results and thus no general decrease of sensitivity of the placenta preparation was caused by the LSD injections. The antagonistic effect of LSD against 5-HT is shown in Fig. 2. In this preparation the vasoconstrictor action of LSD is also illustrated.

Chlorpromazine.—This drug was given in doses of 0.5 to 1 mg. The action of 5-HT and of adrenaline was tested before the administration of

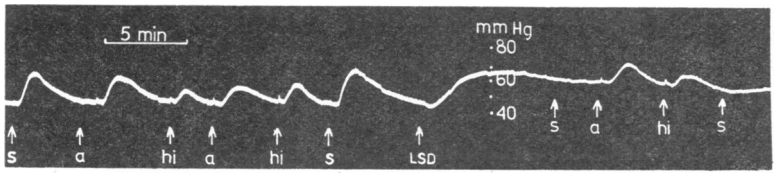

FIG. 2.—Record of umbilical arterial pressure in the isolated perfused human placenta. LSD (25 μg.) completely abolished the action of 5-HT (s, 5 μg.). The effects of adrenaline (a, 20 μg.) and histamine (hi, 25 μg.) were not significantly altered after LSD.

chlorpromazine and at different times afterwards. In the doses used, chlorpromazine completely abolished the effect of 5-HT and adrenaline. The antagonistic effect of chlorpromazine developed gradually and became maximal after 10 to 15 min. The 5-HT effect reappeared after about 30 min., but the action of adrenaline did not reappear during a further hour, after which time the preparation was usually discarded.

In 2 of 3 successful preparations, chlorpromazine in larger doses (2 to 2.5 mg.) had a constrictor action of its own. This effect could still be elicited even if the preparation had been rendered completely insensitive to adrenaline and 5-HT by previous administration of chlorpromazine.

Yohimbine.—When added to the umbilical circulation in doses of 20 to 100 μg. (20 to 50 times the dose of 5-HT required for a good response) yohimbine antagonized the action of subsequently added 5-HT and adrenaline. The anti-5-HT effect of yohimbine was more pronounced than its anti-adrenaline action (Fig. 3).

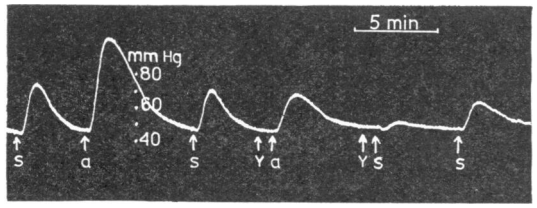

FIG. 3.—Record of umbilical arterial pressure in the isolated perfused human placenta. Yohimbine (Y, 50 μg.) antagonized the action of 5-HT (s, 1 μg.). The effect of adrenaline (a, 10 μg.) was also reduced.

Mescaline.—Mescaline in small doses of 25 to 50 μg. sometimes, but not regularly, caused a slight enhancement of the action of 5-HT. Larger doses from 50 to 300 μg. always had a vasoconstrictor effect. After these large doses the action of 5-HT as well as adrenaline was usually unaffected.

Phentolamine.—In the human placenta 1 to 3 mg. of phentolamine was found to abolish the action of both 5-HT and adrenaline.

Phenbenzamine.—This drug was selected as a representative of antihistamine drugs, and its influence upon the 5-HT effect was studied in 4 placenta preparations. Doses of phenbenzamine (30 to 50 μg.) which completely abolished the action of histamine in 2 of the 4 experiments also completely abolished the effect of 5-HT. In the other 2 experiments the action of 5-HT was not significantly altered after these small doses, which were nevertheless sufficient for histamine blockade. Tests with adrenaline before and after the administration of phenbenzamine were used to confirm that no general decrease of sensitivity had occurred during the experiment.

Reserpine.—The action of reserpine was studied in 4 preparations. Doses of 0.1 to 1.5 mg. had no effect on the perfusion pressure, nor could any certain modifying action on 5-HT be determined. The action of adrenaline also remained unchanged after the addition of reserpine.

Ganglionic Blocking Agents.—The action of tetraethylammonium bromide and hexamethonium bromide was studied in 10 preparations. Both substances were completely inactive in themselves and did not modify the action of 5-HT or adrenaline. The doses used ranged from 1 to 10 mg. for tetraethylammonium and from 1 to 25 mg. for hexamethonium and did not show any toxic effects in these experiments (Fig. 4A).

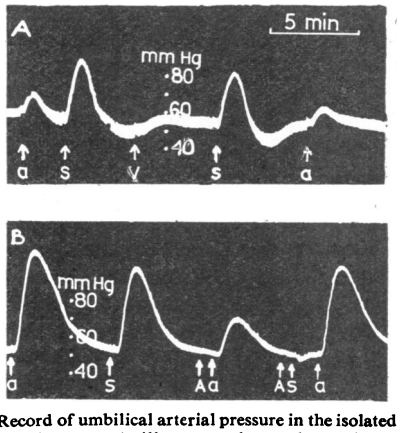

FIG. 4.—Record of umbilical arterial pressure in the isolated perfused human placenta. A illustrates that a large dose of hexamethonium bromide (V, 25 mg.) had no action modifying the effect of 5-HT (s, 2 μg.) or of adrenaline (a, 25 μg.). Note the biphasic response to 5-HT. B illustrates that the ganglion blocking agent trimetaphan (A, 10 mg.) in large doses completely abolished the action of 5-HT (s, 1 μg.), while the adrenaline effect (a, 10 μg.) was less affected.

Another ganglionic blocking agent, trimetaphan, was also tested. Small doses were ineffective, but larger doses (5 to 10 mg.) antagonized the action of subsequently added 5-HT. The effect of adrenaline was similarly, but to a lesser degree, affected by previous injection of trimetaphan (Fig. 4B).

Heparin.—The possible pharmacological action of heparin was studied in 6 preparations in doses ranging from 1 to 30 mg. The action of 5-HT was tested at intervals between injections of increasing doses of heparin. No modifying action on the 5-HT effect could be observed, nor did the general sensitivity of the preparation decrease after these doses.

Discussion

Some previous authors (Reid and Rand, 1952; Freyburger, Graham, Rapport, Seay, Govier, Swoap, and Vander Brook, 1952) have demonstrated that 5-HT shows tachyphylaxis in different preparations. Although tachyphylaxis could not usually be demonstrated in the placenta experiments reported here, it should be pointed out that large doses could not be used because they would cause a complete closure of the vessels.

Some of the antagonists investigated in this study belong to the group of adrenaline antagonists and have been shown to antagonize the action of 5-HT. This is exemplified by phentolamine, which blocks the action of both 5-HT and adrenaline. For both types of action, however, rather large doses were needed (1 to 3 mg.). Phentolamine has previously been found to antagonize the action of 5-HT in the perfused isolated guinea-pig lung (Bhattacharya, 1955). In the placenta preparation dihydroergotamine has also been shown to abolish the action of 5-HT as well as it does the action of adrenaline (Eliasson and Åström, 1955).

Chlorpromazine, which amongst other properties possesses anti-adrenaline effects, has been found to antagonize the action of 5-HT in the placenta. This action of chlorpromazine has previously been reported by Costa (1956) on uterine contractions, by Gyermek, Lázár, and Csák (1956) on the isolated rat uterus, and by Lecomte (1955) on the nictitating membrane of the cat. Potent 5-HT antagonism by chlorpromazine was also demonstrated by Benditt and Rowley (1956) in the rat *in vivo* and on the rat colon *in vitro*.

Yohimbine is a further example of a drug which shows both anti-adrenaline and anti-5-HT properties. As an anti-5-HT compound yohimbine is quite potent in the placenta preparation. The antagonism between yohimbine and 5-HT has been previously studied in other preparations (isolated segments of carotid arteries) by Shaw and Woolley (1953), who first recognized that yohimbine was a structural analogue of 5-HT.

A second group of anti-5-HT compounds are more specific in nature and do not exert any appreciable anti-adrenaline action. This is true especially for LSD, which in small doses completely abolishes the action of 5-HT. Tryptamine in small doses also abolishes the action of 5-HT without significantly altering the adrenaline response. LSD and tryptamine both have a direct vasoconstrictor action if administered in sufficient doses. A constrictor action of LSD on other isolated perfused vascular areas (for example, the rabbit ear) has earlier been demonstrated by Ginzel and Kottegoda (1953) and Savini (1956). The first-mentioned authors also reported that tryptamine was about 10 to 25 times less effective than 5-HT in causing vasoconstriction in the rabbit ear.

Mescaline in small doses caused a slight enhancement of the 5-HT action in some preparations, and larger doses always had a constrictor action on the umbilical vessels. An increase of the action of 5-HT on the isolated uterus after small doses of mescaline has previously been reported by Costa (1956), who also found that large doses of mescaline caused uterine contractions.

Of the ganglionic blocking agents tested, hexamethonium and tetraethylammonium were inactive in the placenta preparation and the actions of 5-HT and adrenaline remained the same even after high doses of these compounds. In the case of hexamethonium, a potentiation of the action of 5-HT was sometimes observed in the isolated guinea-pig ileum by Rocha e Silva, Valle, and Picarelli (1953). A local peripheral vasodilator action with tetraethylammonium chloride was found by Siems and Rottenstein (1950) in the hind limb of the dog. Augmented activity of different vasoactive substances after large doses of tetraethylammonium in dogs, cats, rats, and man has been demonstrated by Page and Taylor (1950).

In the group of ganglionic blocking agents tested, only trimetaphan was found to be effective in the placenta preparation. In large doses it antagonized the action of 5-HT and to a lesser degree that of adrenaline. Evidence that trimetaphan has direct peripheral actions, besides its ganglionic blocking properties, has earlier been presented by McCubbin and Page (1952).

Reserpine, which has been reported by Costa (1956) to antagonize 5-HT induced uterine contractions, was found to be ineffective in the

placenta preparation. The same was true also for heparin even in very high doses. Heparin was studied in this investigation, since it had previously been demonstrated that heparin exerts an antagonistic effect on the action of 5-HT *in vivo* and *in vitro* (Smith and Smith, 1955). An antagonism of this kind would be of great interest in clinical cases of embolism or thrombosis. A local release of 5-HT could, by way of vasoconstriction, contribute to the impairment of the local circulation in such patients (Comroe, van Lingen, Stroud, and Roncoroni, 1953).

The results obtained in this investigation on a non-innervated preparation speak in favour of a direct mode of action of 5-HT on the smooth muscles of the vessels. The antagonists included in this study exerted their action in this preparation in the same manner as has previously been demonstrated in different innervated organs. This indicates also that the mechanism of antagonism is independent of the presence of nervous structures.

The fact that 5-HT has strong vasoconstrictor actions in different local vascular regions, and the observation that the mechanism of antagonism also seems to be located at the smooth muscles of the vessels, are of particular interest for the study of psychotic effects of some of these compounds. Even if the results obtained in the placenta and other isolated vascular beds cannot directly be applied to the cerebral vessels it seems worth considering that some of the changes observed in psychic functions, particularly after local administration of the drugs into the ventricles of the brain, might be produced as a result of altered blood supply to different central nervous structures.

If the results obtained in this study are compared with those of Ginzel and Kottegoda (1953), who studied the effect of 5-HT and some of its antagonists on the lung circulation, great similarity is revealed. This illustrates further the point brought out by Euler (1938), that striking similarity seems to exist between the pharmacological responses of the umbilical and the lung vessels.

This investigation was supported by a grant from Karolinska Institutets Reservationsanslag. For kind co-operation in obtaining the placentae we owe our thanks to the staff at the Departments of Obstetrics and Gynaecology of Karolinska and Sabbatsberg's Hospitals. We are also grateful to Mr. Nils-Åke Persson for technical assistance.

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